

The University of Chicago

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OF EXOERYTHROCYTIC STAGES

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UNITED STATES AND MEXICO

A DISSERTATION SUBMITTED TO THE FACULTY OF THE DIVISION OF THE BIOLOGICAL  
SCIENCES IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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# A SAURIAN MALARIAL PARASITE, *PLASMODIUM MEXICANUM*, N. SP., WITH BOTH ELONGATUM- AND GALLINACEUM-TYPES OF EXOERYTHROCYTIC STAGES

PAUL E. THOMPSON

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## INTRODUCTION

The history of malaria reveals that studies of infections in various species of experimental hosts have contributed to the fundamental understanding of this disease and that there have been certain special advantages associated with the use of each type of animal. In view of past experience it would seem that as many types of malarial infections as possible should be studied. Except for morphological descriptions of species of parasites, saurian malaria has been neglected. The present studies, together with those of the following paper, were projected with the idea that the lizard may have certain advantages as an experimental animal and that knowledge of the nature of malarial infections in cold-blooded animals should contribute to the understanding of various aspects of malariology. The finding that the species of saurian malarial parasite described here is capable of producing both *elongatum*- and *gallinaceum*-types of exoerythrocytic stages comes at an especially opportune time by contributing to the understanding of the significance of exoerythrocytic stages in the schizogonous cycle of malarial parasites.

## MATERIALS AND METHODS

*The parasite.*—The strain of parasites

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was isolated from *Sceloporus ferrari-perezi*, obtained in Michoacan, Mexico.

*The lizards.*—The experimental animals consisted of lizards in which no natural infections have been observed and they were obtained from different parts of the United States and Mexico in which saurian malaria has not been found. The one exception to this was the Mexican horned lizard, *Phrynosoma asio*, obtained from Colima, Mexico, but in this species *Plasmodium* has not been encountered. The other lizards in which experimentally-induced infections have been studied included the collared lizard, *Crotaphytus collaris*; the common horned lizard, *Phrynosoma cornutum*; the Texas spiny swift, *Sceloporus olivaceous*; and the common fence lizard, *Sceloporus undulatus*, obtained from North Carolina and Illinois. The fact that various other parasites occur naturally in lizards has necessitated that great care be taken not to use any lizards that harbor parasites which may be confused with any stages of *Plasmodium*.

*Care of the lizards.*—It is a matter of common observation on the part of herpetologists and others interested in maintaining lizards in captivity that some animals fail to become adjusted to laboratory conditions and die within a few weeks. In the early part of this work considerable difficulty was encountered in maintaining the lizards long enough for experimental studies. This problem has not been solved completely, but the following conditions have made possible the maintenance of at least two-thirds of the specimens for



periods varying from 3 months to over a year. (The exception to this has been *Phrynosoma*, a smaller proportion of which have been maintained in the laboratory.) Either wire or glass cages with screen tops and a layer of sand in the bottom were used satisfactorily. Artificial heat and light were provided by burying a small light bulb (10- or 15-watt) in the sand at one end of the cage and by using a larger bulb (60- or 100-watt) on top of the cage. This gave a temperature gradient ranging from approximately 35 C to near room temperature. The light bulbs were turned off at night in an effort to approximate natural conditions with reference to heat and light. A high temperature and a bright light were found to encourage the lizards to eat voluntarily. Most of the species used in this study fed sparingly when the temperature was below 21 C. The food for most of the lizards consisted mainly of mealworms (larval stages of *Tenebrio molitor*). Forced feeding with beef, liver, or mealworms and the giving of water by means of a medicine dropper made it possible to maintain a majority of the specimens of *C. collaris* for a year.

*Technics.*—Blood smears usually were most satisfactorily made by clipping off the end of a toe. However, the tail of the horned toad is sufficiently vascular to serve as a source of blood for smears and for transferring the strain. In transferring the parasites, blood was taken from the toe; in the case of the larger lizards, it was taken from the heart with a 27-gauge hypodermic needle attached to a syringe containing a small amount of heparinized saline solution. Many of the lizards were bled from the heart repeatedly without appreciable ill effects. Inoculations were made by the intraperitoneal, intravenous, intracardial, intramuscular, or subcutaneous routes.

Both dry- and wet-fixed smears were made. Dry smears were made by smearing the blood in the usual manner, allowing it to dry in the air, fixing in absolute methyl alcohol for one minute, and staining with MacNeal's tetrachrome or Giemsa's stain. Satisfactory results were secured by using either of these, except in the staining of *P. mexicanum*. The occurrence of this parasite in basophilic cells made it more desirable to use Giemsa's stain as an aid in the recognition of the smaller stages. This stain seemed to have a greater affinity for the nuclei of the parasites than tetrachrome, and therefore facilitated distinguishing these nuclei from red granules present in the cytoplasm of many uninfected basophilic cells.

Wet-fixed smears and tissue sections were fixed in Zenker-formol and stained with hematoxylin and eosin-azur II by Maximow's method, as employed by Huff and Bloom (1935).

*Enumeration of parasites.*—The degree of parasitemia has been expressed in terms of the number of parasites per 10,000 blood cells. Enough parasites were counted in each case to keep the probable error under 10%. The parasitemia was expressed in terms of the total number of blood cells rather than in terms of erythrocytes because of the extensive occurrence of *P. mexicanum* in a wide variety of blood cells.

*Differentiation of blood and connective tissue cells.*—The occurrence of myelopoiesis and lymphopoiesis in the same tissues of the lizard has increased the difficulty of distinguishing between certain types of blood and connective tissue cells. In general, the differential criteria and terminology employed by Huff and Bloom (1935) in identifying myeloid, lymphoid, and connective tissue cells of the canary have been employed in identifying corresponding cells in the lizard. The descriptions of Jordan and



Speidel (1929) of various types of blood and connective tissue cells in the horned toad, *Phrynosoma solare*, have also been followed in making these distinctions.

#### EXPERIMENTAL STUDIES

##### *Discovery and isolation of the parasite.*

—The presence of malarial parasites in *Sceloporus ferrariperezi* procured from Mexico was reported by Huff (1942). Since then, 61 of these lizards have been examined, 23 of which have been found to harbor malarial parasites characterized by the same type of gametocytes. A strain of parasites was isolated from one of these hosts by inoculating blood into *Crotaphytus collaris* (Thompson and Huff, 1941). This strain, which has been maintained for over a year in several species of lizards, has been found to consist of a single species, here described as *Plasmodium mexicanum*, n. sp.

*Host susceptibility.*—In an effort to find a highly susceptible host suitable for experimental studies, several species of uninfected lizards were inoculated. The species of lizards inoculated, the number of lizards of each species, and the results of the inoculations are given in table 1.

TABLE 1.—Results of the Inoculation of Various Species of Lizards with *P. mexicanum*

| Family and Species of Lizard | Number Inoculated  | Number Infected |
|------------------------------|--------------------|-----------------|
| Family Helodermatidae        |                    |                 |
| <i>Heloderma horridum</i>    | 1 (2 inoculations) | 0               |
| Family Iguanidae             |                    |                 |
| <i>Anolis carolinensis</i>   | 1                  | 0               |
| <i>Crotaphytus collaris</i>  | 25                 | 12              |
| <i>Phrynosoma asio</i>       | 4                  | 4               |
| <i>Phrynosoma cornutum</i>   | 14                 | 11              |
| <i>Sceloporus olivaceus</i>  | 8                  | 7               |
| <i>Sceloporus undulatus</i>  | 8                  | 6               |
| Family Scincidae             |                    |                 |
| <i>Eumeces fasciatus</i>     | 1                  | 0               |
| <i>Eumeces laticeps</i>      | 2                  | 0               |
| <i>Eumeces obsoletus</i>     | 1                  | 0               |

Infections were established only in lizards belonging to the family Iguanidae, which is also the family that includes the natural host, *Sceloporus ferrariperezi*. Lizards of the species *Crotaphytus collaris* were the least sus-

ceptible of the 5 species of lizards infected, whereas *Phrynosoma cornutum*, *P. asio*, *S. undulatus*, and *S. olivaceus* were highly susceptible.

In experiments on *C. collaris*, inoculations with blood or tissues (liver and bone marrow) suspended in saline were given by the intravenous, intraperitoneal, intracardial, or subcutaneous routes. Intravenous inoculations, which were made into a toe vein, and intracardial inoculations were found to be the most efficient means of transferring the strain. In the other species of lizards all inoculations were given intraperitoneally, except for one intracardial inoculation into *S. olivaceus*. Although *Phrynosoma cornutum*, *P. asio*, *S. undulatus*, and *S. olivaceus* were relatively susceptible to *P. mexicanum*, inoculation of rather large numbers of parasites was necessary to establish an infection in these lizards as well as in *C. collaris*. Patent infections were not produced occasionally, even when substantial inoculums were given.

*Incubation period.*—Data on the incubation period, length of the acute period, latency, and relapse in the various species of hosts are given in figure 1. Due to the fact that smears were made at intervals of several days, the exact length of the incubation period was not determined accurately in many cases. Although the length of the incubation period varied considerably for different lizards of the same species, the average length seemed to vary significantly with the species of host. The average time required for infections to become microscopically patent was: for *C. collaris*, 30 days; *Phrynosoma cornutum*, 25 days; *S. undulatus*, 20 days; *P. asio*, 19 days; and *S. olivaceus*, 17 days. Variation in the length of the incubation period within each species of host was probably mainly due to differences in the number



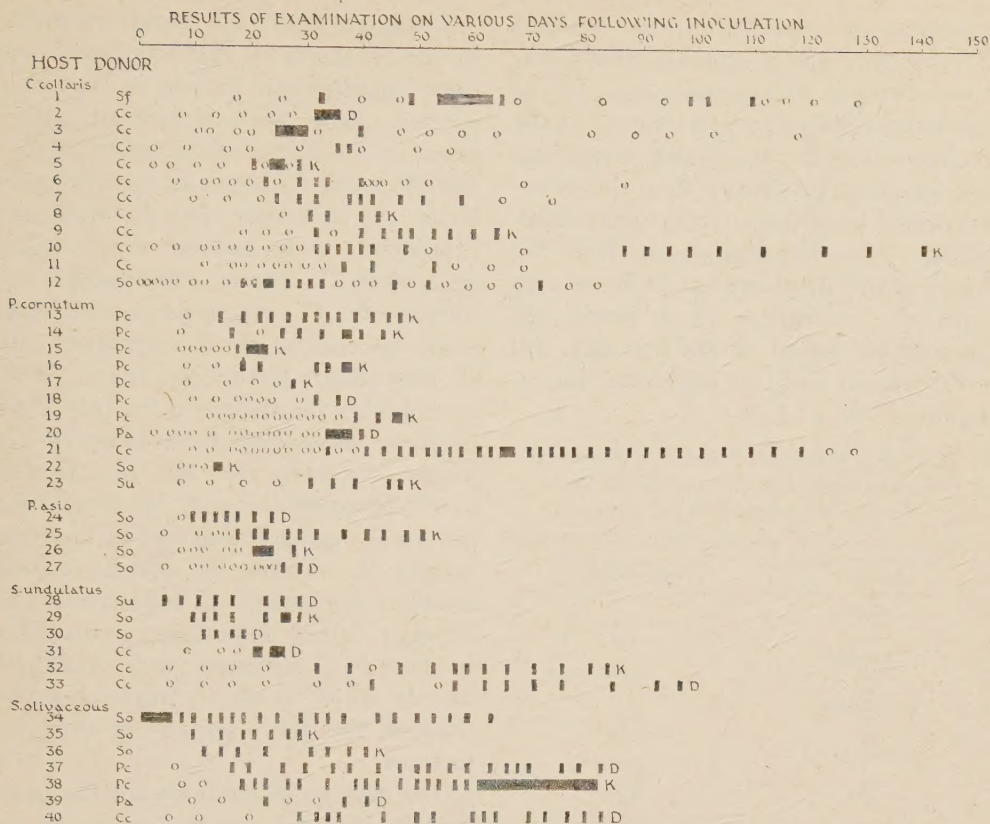


Fig. 1.—Species and numbers of lizards experimentally infected with *P. mexicanum*, sources of the inoculums, time required for the infections to develop, and the length of time parasites were observed in the circulating blood. (Inoculums given intraperitoneally, except nos. 1 and 3, given intravenously, and nos. 12 and 34, intracardially.)

O=parasites not observed within 5 to 10 minutes or longer; shaded area—from 1 to many parasites observed, length of area indicates number of successive days parasites observed.

K=Killed      Sf=*S. ferrariperezi*      Cc=*C. collaris*      Pc=*P. cornutum*      Pa=*P. asio*  
D=Died      Su=*S. undulatus*      So=*S. olivaceous*

of parasites injected. In *S. undulatus* and *S. olivaceous* there was an indication that the incubation period was shorter when parasites were transferred within the same species of host, but in some of these cases the number of parasites may have been greater than was generally used. One *S. olivaceous* (No. 34) was inoculated intracardially with blood from an infected *S. olivaceous* with an inoculum of such size that there was an average of two parasites per 100 blood cells immediately afterwards in blood taken from the toe of the recipient.

Parasites were found in the blood the next day and constantly for the next 67 days, at which time the lizard died. This infection remained at a very low level even though a large number of parasites had been injected.

In experiments on *C. collaris* the length of the incubation period did not seem to depend upon the method of inoculation, the period being approximately equally variable when inoculations were given by the intravenous, or by the intraperitoneal route. In a single intracardial inoculation (No. 12), the

inoculum of blood from an infected *S. olivaceus* was of such size that parasites occurred in approximately 1% of the blood cells taken from the toe of the recipient, but the incubation period was not significantly shorter than the shortest period following intraperitoneal inoculation. Parasites disappeared from the blood of this lizard within 24 hours and, with the exception of a single old gametocyte found on the 5th day, did not reappear until 19 days after inoculation.

TABLE 2.—Maximum Numbers of Parasites Observed in the Experimental Hosts Most Heavily Infected with *P. mexicanum*

| Species and Number of Lizard | Parasites per 10,000 Blood Cells |
|------------------------------|----------------------------------|
| <i>C. collaris</i>           |                                  |
| 2                            | 165                              |
| 8                            | 238                              |
| <i>P. cornutum</i>           |                                  |
| 13                           | 84                               |
| 14                           | 392                              |
| <i>S. undulatus</i>          |                                  |
| 29                           | 2812                             |
| 31                           | 291                              |
| <i>S. olivaceus</i>          |                                  |
| 37                           | 6900                             |
| 38                           | 6934                             |
| 40                           | 4100                             |

The irregularity with which parasites were observed at the beginning and end of the acute periods in various lizards was due to the relatively slow rate at which the infections developed and subsided. Probably for this reason a single parasite would be encountered on some days, and not on others, although actually they may have been approximately as numerous throughout the period of examination.

*The parasitemia.*—The types of infection experimentally produced varied from those barely detectable microscopically to heavy infections. Among the factors contributing to this variability were differences in the susceptibility of the various hosts, the number of parasites injected, and the length of time the infections were studied. The heaviest infections were produced in the lizards listed in table 2.

Although there was much variation in the intensity of the infections produced in different lizards of the same species, there were significant differences in the degree of parasitemia produced in the 5 species of experimental hosts. *S. olivaceus* and *S. undulatus* developed much heavier blood infections than *C. collaris*, *Phrynosoma cornutum*, and *P. asio*. It is possible that some specimens of *P. cornutum* and *P. asio* might have been found to be more highly parasitized if the infections in these hosts had been followed for longer periods of time, but the infection in *P. cornutum* (No. 21) remained at a low level during the period of approximately 4 months in which it was studied. Apparently *C. collaris* will not develop blood infections comparable to those in *S. olivaceus* since 6 of these lizards failed to do so when observed through the acute periods of their infections.

The courses of infection for the most heavily infected lizards are shown in figure 2. The infection in *S. olivaceus* (No. 38) is especially interesting because the marked decrease in number of parasites after the 70th day suggests that acquired immunity was beginning to operate efficiently. The host at this time had become very anemic, due partly to repeated bleeding, and it seems likely that its debilitated condition may have interfered with the immune response so that parasites did not continue to decrease.

The degree of injury resulting from infections with *P. mexicanum* cannot be evaluated satisfactorily from mortality figures, due to wide variations in the length of time both infected and uninfected lizards survived under the experimental laboratory conditions.

*Length of the acute period, latency, and relapse.*—In 6 specimens of *C. collaris* the acute periods varied from 11 to 32



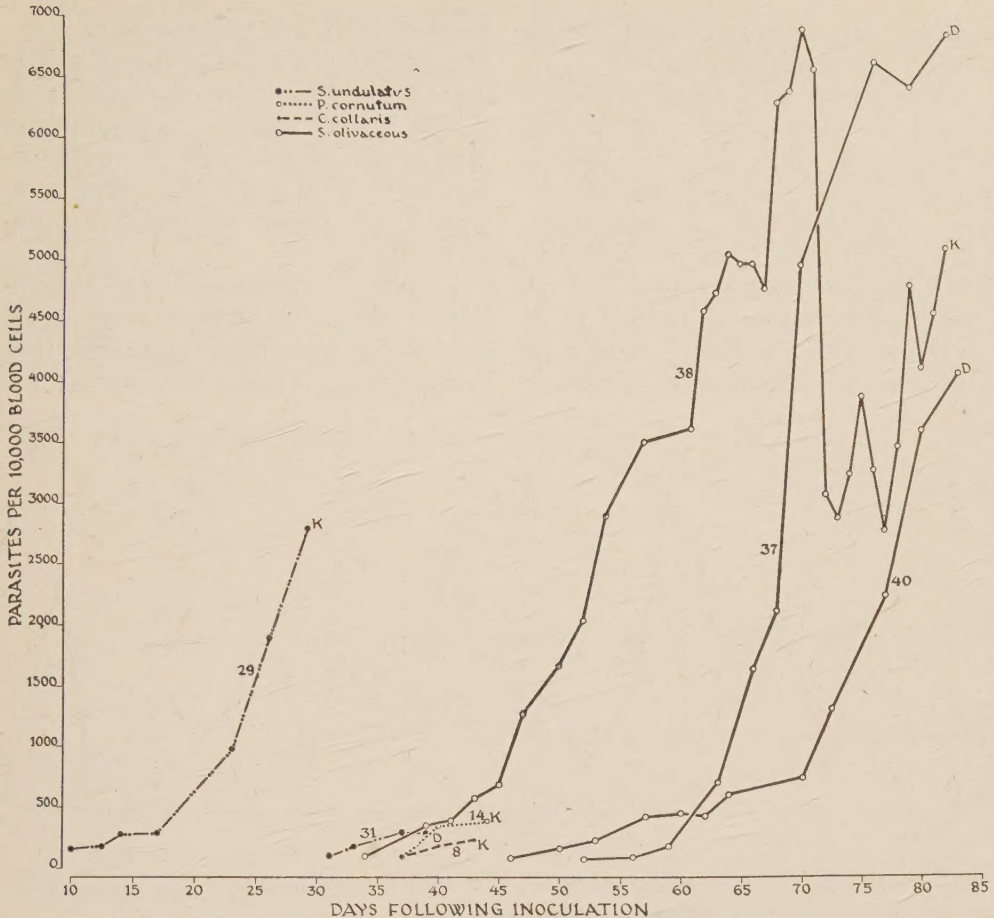


Fig. 2.—Courses of infection with *P. mexicanum* in the experimental hosts having the greatest parasitemia.

days. This was significantly shorter than the acute periods in the other species of hosts, although the exact length of this period was determined only in the case of *Phrynosoma cornutum* (No. 21). The infection in this host lasted approximately 85 days. In *C. collaris* the blood became negative either as the result of spontaneous eradication of the parasites or the development of a latent period. The data from lizards 1 and 10 indicate that a latent period may be followed by a relapse. In No. 1 the appearance of parasites on the 99th and 102nd days following inoculation is probably an example of a relapse. Al-

though the infection in No. 10 included acute, latent, and relapse periods, it may be atypical because of severe anemia. This was partly induced by repeated bleeding, since a total of 3.2 cc of blood was taken between the 45th and 128th days following inoculation for infecting other lizards. The severity of this treatment may be appreciated when it is pointed out that this lizard weighed only approximately 25 g. The severe debilitation associated with extreme anemia may have altered the normal immune reactions of the host at this stage in favor of greater susceptibility to the parasite. The infection was



approximately as heavy during the relapse as during the acute period.

*Synchronism or periodicity.*—The asexual reproduction was studied in *C. collaris* in an effort to determine the length of the asexual cycle and the degree of synchronism or periodicity. Smears were made from lizard No. 10 at 6-hour intervals for 9 days, and the percentage of parasites having 16 or more nuclei or actually undergoing schizogony was determined. (Segment-

to 21 or 24 C. The percentages of parasites undergoing segmentation or having more than 16 nuclei are shown in figure 3. It is evident that the infection in this host is asynchronous and is not characterized by high peaks of maximum reproduction during the period of observation.

Exploratory tabulations and the apparent absence of synchronism as revealed by inspection suggested that similar findings would characterize the



Fig. 3.—Asexual reproduction of *P. mexicanum* in *C. collaris* as indicated by the percentage of segmenters and schizonts with more than 15 nuclei for 9 days.

ers of *P. mexicanum* produce an average of 19 merozoites in this host.) The percentage of segmenters or mature schizonts was determined by examining 50 parasites in each smear. (Parasites having fewer than 3 nuclei were not counted because of the difficulty in accurately recognizing the smaller stages when they occur in basophilic cells stained with tetrachrome stain.) During the entire course of the infection the lizard was kept under conditions approximating the normal conditions of light and temperature. During the day the cage was well-lighted and there was a temperature gradient ranging from approximately 27 to 35 C; at night the cage was dark and the temperature fell

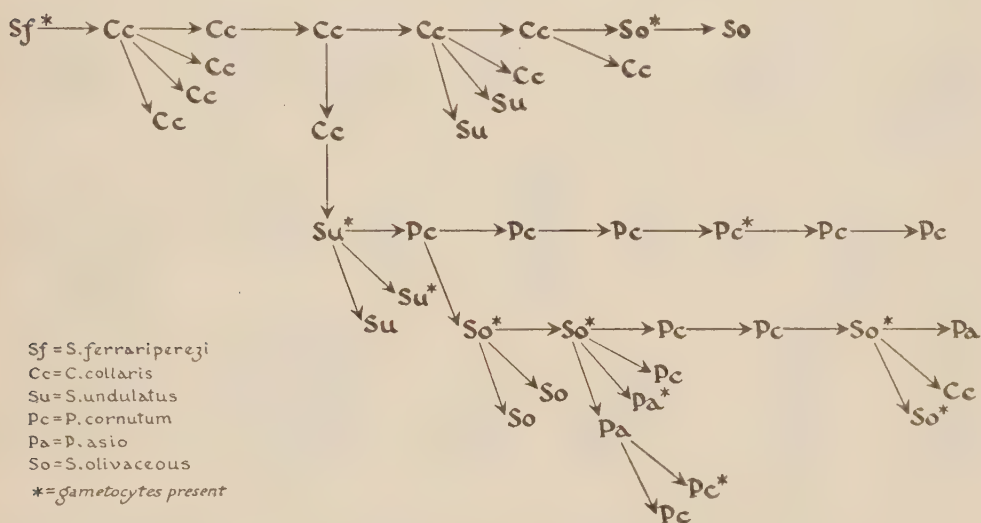
infections of *P. mexicanum* in the other experimental hosts.

*Gametocyte production.*—One of the most interesting observations regarding the behavior of *P. mexicanum* in various species of hosts has been the variation in gametocyte production. The blood infection in the natural host, *Sceloporus ferrariperezi*, from which the strain was isolated, consisted primarily of gametocytes. In contrast to the abundance of gametocytes in *S. ferrariperezi*, these stages have not been found in 12 specimens of *C. collaris* in which the strain was under observation for over a year, with the exception of a single probable pregametocyte in lizard No. 10 (Pl. 1, fig. 7). During this time over 100 blood



smears from these hosts were examined thoroughly, approximately one-third of which were used for number counts in the study of the asexual reproduction and the degree of parasitemia. Many parasites, therefore, were studied in each smear. In addition, in several of the lizards, extensive studies were made upon the spleen, liver, bone marrow, heart, kidneys, lungs, intestine, skeletal muscles, subcutaneous tissues, and brain. The strain in this host apparently

*Phrynosoma cornutum*, *P. asio*, *S. undulatus*, and *S. olivaceus*, there was much variation in the numbers occurring in the lizards of different species. Extremely small numbers have been seen in 2 specimens of *Phrynosoma cornutum* (Nos. 14 and 21) and in one *P. asio* (No. 24). Only one *S. undulatus* (No. 29) produced gametocytes, but the latter became numerous. Five specimens of *S. olivaceus* (Nos. 34, 35, 37, 38, 40) produced many gametocytes. The fail-





variety of blood cells was parasitized. With the establishment of infections in several species of hosts it has been possible to make preliminary observations on the comparative histopathology of the parasite, with special emphasis upon the types of cells infected. For sake of convenience in presentation these observations will be described on a comparative basis under 2 divisions: namely, those from peripheral blood smears, and those from tissue sections and wet-fixed, impression smears of tissues.

*Types of cells infected in the peripheral blood.*—Both erythrocytic and exoerythrocytic stages occurred in all species of experimental hosts. In each experimental host a majority, if not all, types of blood cells were infected. Representative types of parasitized cells are

shown in Pl. 1. These include all cells in the erythrocyte series—erythrocytes (fig. 6), normoblasts (figs. 4, 5, 8, 9), polychromatophil erythroblasts (figs. 7, 10), and basophil erythroblasts (fig. 11); granulocytes (fig. 21) and myelocytes (fig. 19); lymphocytes (figs. 12, 13, 14, 16) and monocytes (fig. 17); thrombocytes (fig. 15); and circulating macrophages (fig. 22). Several smears from naturally infected *S. ferrariperezi* containing gametocytes similar to those of *P. mexicanum* have been examined in an effort to determine whether exoerythrocytic stages occur in the natural hosts. The parasites in all of these smears were in cells possessing hemoglobin, but it should be emphasized that few asexual stages were encountered. The great majority of the gametocytes occurred in normoblasts

#### EXPLANATION OF PLATE

Plate 1.—Malarial parasites and unidentified bodies in naturally infected and experimentally infected lizards.

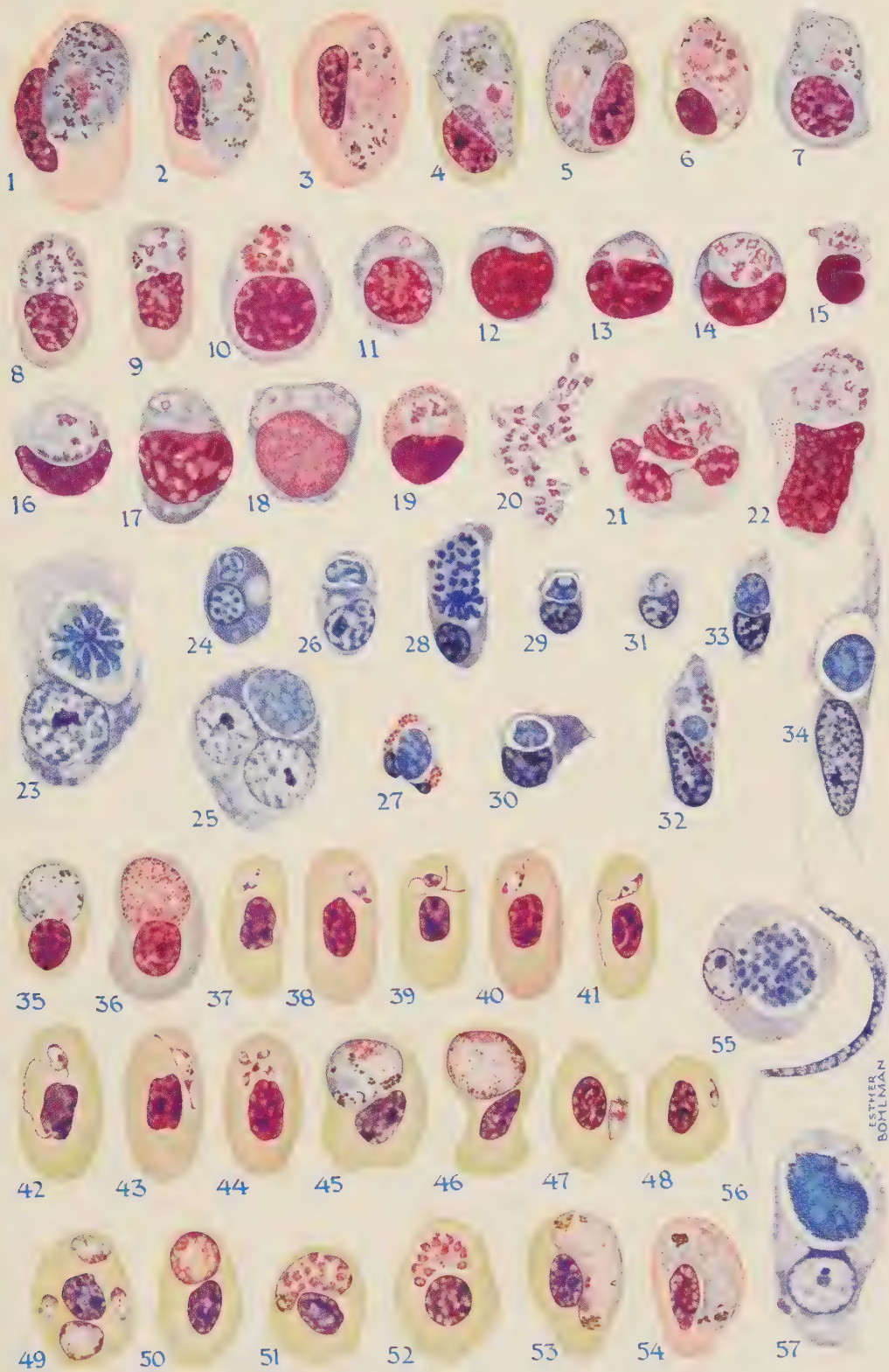
Figures 1–22, 35–54, and 56 are from air-dried blood smears stained with MacNeal's tetrachrome or Giemsa's stain. 23–34, 55 and 57 are from smears or sections of tissues wet-fixed in Zenker-formol and stained by Maximow's hematoxylin eosin-azur method (23–25, from smears; others from sections). Magnification  $\times 1370$ . Drawn by Miss Esther Bohlman.

Figs. 1–34.—*Plasmodium mexicanum*, n. sp. 1 and 2, macrogametocytes; 3, microgametocytes in normoblasts from natural host, *Sceloporus ferrariperezi*. 4 and 5, macrogametocytes in normoblasts; and 6, microgametocyte in erythrocyte from *S. undulatus*. 7, probably pregametocyte in polychromatophil erythroblast; 8 and 9, schizonts in normoblasts; 7–9 from *Crotaphytus collaris*. 10, segmenter in polychromatophil erythroblast; and 11, trophozoite in basophil erythroblast from *Phrynosoma cornutum*. 12 and 13, trophozoites in lymphocytes; 14 and 16, schizonts in lymphocytes; 15, schizont in thrombocyte; 17, 18 and 19, trophozoites in a monocyte, in a probable stem cell and in a myelocyte, respectively; and 20, merozoites: 12–20 from *C. collaris*. 21, trophozoite in granulocyte (probably a heterophil); 22, schizont in circulating macrophage; 23, segmenter in rounded up reticular cell; 24, trophozoite in plasma cell; 25, schizont in binucleate reticular cell; 26, trophozoite in lymphocyte; 27, schizont in heterophil; 28, segmenter in a probable early plasma cell; 29, trophozoite in a probable basophil erythroblast; 30, trophozoite in a probable haemocytoblast: 21–30 from *Phrynosoma cornutum*. 31, trophozoite in a circulating lymphocyte; 32 trophozoite in a probable eosinophil myelocyte; 33, schizont in a probable lymphocyte; 34, schizont in a reticular cell: 31–34 from *C. collaris*. (1–22 from the blood; 23–26, 28–30 from the spleen; 27, 32–34 from the bone marrow; and 31 from the liver.)

Figs. 35–44.—*Plasmodium rhadinurum*, n. sp. in erythrocytes of *Iguana iguana rhinolopha*. 35, macrogametocyte; 36, microgametocyte; 37–43, trophozoites; and 44, segmenter.

Figs. 45–54.—*Plasmodium floridense*, n. sp. in erythrocytes of *S. undulatus*. 45, macrogametocyte; 46, microgametocyte; 47–49, trophozoites; 50 and 51, schizonts; 52, segmenter; 53, immature microgametocyte; 54, immature macrogametocyte.

Figs. 55–57.—Unidentified bodies. 55, coccidia-like segmenter in hepatic cell; 56, sergentella-like organism in circulating blood from *Phrynosoma cornutum*. 57, bodies in hepatic cell of *S. undulatus* which resemble inclusion bodies of mouse pneumonitis and related viruses.







in these smears, and it seems very probable that *P. mexicanum* may invade a variety of blood cells in *S. ferrariperezi*.

The greatest variation in the behavior of the parasites in different species of hosts has been in the quantitative dis-

tribution of parasites in the different species of hosts, and except in the case of *S. undulatus*, more than a single lizard is represented.

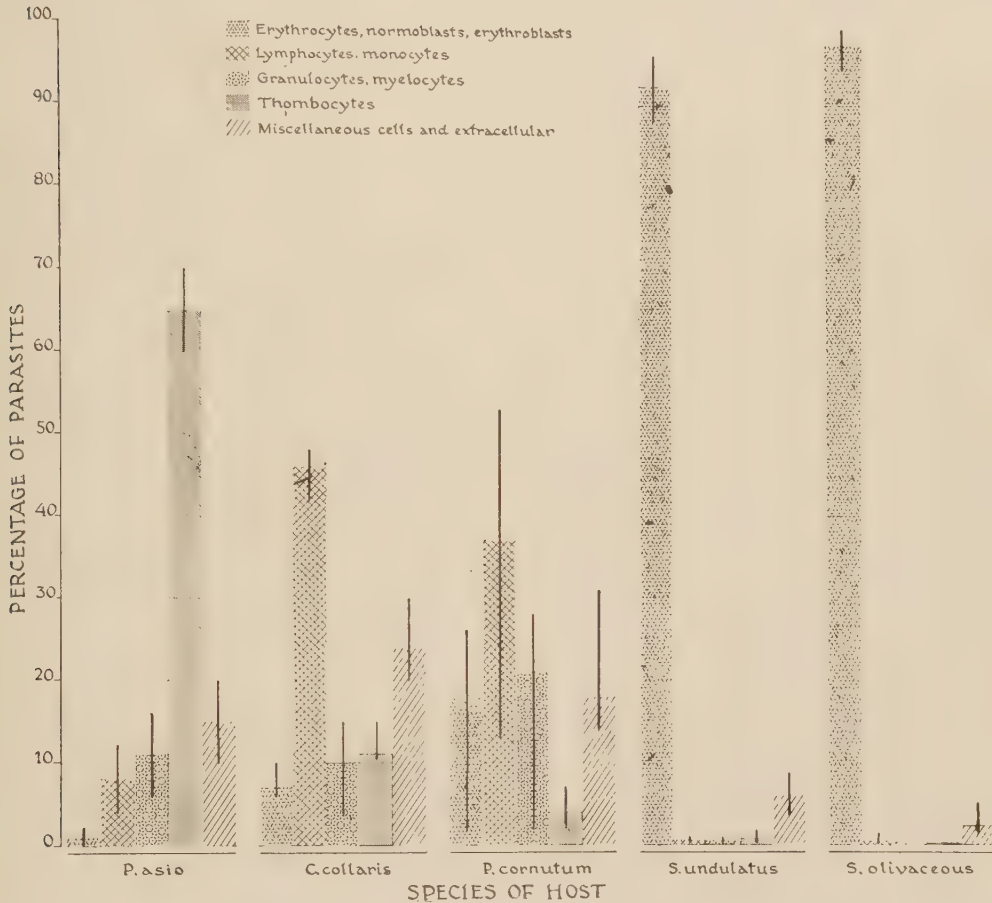


Fig. 5.—Percentage distribution of *P. mexicanum* in various groups of blood cells in 5 species of experimental hosts. (The mean percentages are indicated by the bars, and the maximum and minimum percentages are indicated by the lines in the bars.)

tribution of parasites in the different types of cells rather than in the types of cells parasitized. This is shown in figure 5, which gives the percentage of parasites in various types of circulating cells. The average percentage of parasites in the different groups of cells is indicated by the length of the bars in the graph, and the range of percentages

The number of parasites counted in each species of host was: *C. collaris*, 150; *P. cornutum*, 249; *P. asio*, 100; *S. undulatus*, 450; *S. olivaceous*, 1,000. The greatest variation in the cellular distribution of parasites in different smears from the same lizard, or in smears from different lizards of the same species, occurred in *Phrynosoma cornutum*. In



*C. collaris*, *P. cornutum*, and *P. asio*, the majority of the parasites occurred in cells lacking hemoglobin, while the converse was true in *S. undulatus* and *S. olivaceous*. These data represent only in a general way the distribution of parasites, since the smears from which the tabulations were made represent infections of varying duration and intensity. Furthermore, variations in the number of cells of each type have not been considered. Variations of this sort occur in anemia and probably there are wide variations in the number of circulating leucocytes. Examples of factors that may have produced variations in the number of circulating leucocytes were observed in *P. cornutum*, many of which harbored large numbers of round worms in their stomachs, and upon histological examinations were found to be infected with coccidia-like parasites. It seems possible that such factors as these, through alterations in the numbers of various types of circulating cells, may account in part for the wide variation in the cellular distribution of *P. mexicanum* in this host.

*Cells infected and distribution of parasites in the tissues.*—Study of tissue sections and wet-fixed impression smears from various organs of all species of experimentally infected lizards has revealed that in addition to the exoerythrocytic stages observed in the circulating blood, there are stages in certain fixed cells similar to the gallinaceum-type of exoerythrocytic stages (Porter, 1942) described for several species of avian malarial parasites. While these stages have been observed in all hosts it is not possible at this time to describe in detail the distribution of parasites in *P. asio*, since extraneous "parasites" of unknown identity have been encountered in the tissues examined. Because of the uncertainty in distinguishing these from the malarial parasites, all

tissues harboring such extraneous forms have been omitted in the following description. The extraneous "parasites" are described in the following paper (p. 75).

In tissues from *C. collaris*, *P. cornutum*, and *S. olivaceous* the numbers of exoerythrocytic stages have varied widely. They were very numerous in *C. collaris* and *P. cornutum* and comparatively rare in *S. olivaceous*; with respect to the range of cells parasitized, however, there has been much less variation. In addition to those listed from the circulating blood, the following types of cells were parasitized in the tissues of each of these lizards: reticular cells, littoral cells of the spleen and liver, hemocytoblasts, tissue macrophages, true endothelial cells of capillaries, of other small blood vessels, and of the interstices between the trabeculae of the cardiac muscle. In addition to these, parasites have been found occasionally in certain species of hosts in cells that thus far have not been observed to be infected in other hosts.

It is possible that the percentage of parasites in each of the various blood and connective tissue cells and the proportion in various organs vary with the stage of the infection. Apparently, there may be at least two possible factors which affect the distribution of parasites. First, if the immune response in saurian malaria is similar to that observed in avian malaria by Cannon and Taliaferro (1931), and in simian malaria by Taliaferro and Cannon (1936) and Taliaferro and Mulligan (1937), during the period of acquired immunity we should expect to find a higher percentage of parasites in phagocytic cells than during the time when only natural immunity is operating. A second possible factor affecting the distribution of parasites is suggested by the findings of Porter (1942) in which there were

changes in the distribution of exoerythrocytic stages of *P. cathemerium* of birds during various stages of sporozoite-induced infections. A big obstacle in presenting the tissue distribution of parasites in various species of hosts on a comparative basis arises from the difficulty of determining comparable stages of the infections, since the course of the infection varies in different hosts. The following description, therefore, probably indicates only general differences in the behavior of *P. mexicanum* in the various hosts.

In *C. collaris*, parasites were much more numerous in the spleen and other highly vascular tissues than in the peripheral blood, this distribution reflecting the marked affinity of *P. mexicanum* for various types of fixed cells in this host. A large proportion of the parasites occurred in fixed connective tissue cells. The spleen contained the greatest number per unit area, and here most of the parasites were in reticular cells, macrophages, and the littoral cells of the venous sinuses. Segmenters in these cells produced several times as many merozoites as in most of the circulating blood cells. In avian malaria, the exoerythrocytic schizont of the *gallinaceum*-type also produces much larger numbers of merozoites than are produced by an erythrocytic segmenter (Porter and Huff, 1940). Next to the fixed connective tissue cells, the most frequently parasitized cells were lymphocytes and erythroblasts, which were difficult to differentiate accurately when the parasite filled the cytoplasm and distorted the nucleus. In addition to these cells, parasites occurred frequently in hemocytoblasts, myelocytes, and heterophils (pseudo-eosinophils). Next to the spleen the greatest concentration of parasites occurred in the tissues possessing the largest number of blood vessels. Parasites were found in

large numbers in the endothelial cells and pericytes of capillaries throughout the body. In the most heavily infected animals, parasites could be seen in virtually every microscopic field when examining the more vascular areas of the lungs, kidneys, heart, lamina propria of the intestine, meninges, and subcutaneous tissues. Some such parasites in various stages of development in the capillary endothelium are shown in Pl. 2, figure 2, which was drawn from the arachnoid-pia mater (lizard No. 2). The tendency of *P. mexicanum* to parasitize true endothelium in *C. collaris* is such that more parasites occur per microscopic field in the most vascular parts of the lungs and kidneys than in the bone marrow and liver. Many parasites were found in the endothelial cells and adventitial cells of the venules. These were especially numerous in lizard No. 8, which had the heaviest blood infection seen in this species of host and was killed on the 12th day of the patent infection. The extent to which small portal venules in the liver of this host contained parasites is shown in Pl. 2, figure 1. In the heart, parasites occurred in the cells lining the interstices of the myocardium (Pl. 2, fig. 4). In lizards the blood supply for cardiac muscle is provided by the direct circulation of blood between the muscle trabeculae rather than by capillaries, as in the higher vertebrates. The cells lining these intertrabecular spaces are regarded as of the true endothelial type (Bloom, 1943). In the bone marrow, most of the parasites were in reticular cells. The extent to which these cells may be parasitized is shown in Pl. 2, figure 3, which shows an area of reticular cell hyperplasia in the endosteum of lizard No. 2, which had died on the 5th day after parasites appeared in the blood. The leg of this animal had been accidentally fractured approximately 3





PLATE 2

weeks previously. Normally, the endosteum contains only 1 or 2 layers of reticular cells. It is not known definitely that this tissue came from the fractured leg, but in all probability it did, since such hyperplasia is associated with repair of a fracture, and reticular cell hyperplasia of this sort has not been observed in tissues from other infected specimens of *C. collaris*. In striking contrast with the large number of parasites in the area of reticular cell hyperplasia, there were few in the bone marrow proper. In the liver, parasites occurred mainly in the cells lining the sinusoids. Occasionally small parasites were seen in Kupffer cells, but due to the large amount of pigment from the skin, mesenteries and other tissues, it was difficult to detect parasites in these cells. The propensity of the parasites to occur in fixed cells in this host suggested the possibility that the infection in the tissues may persist after the disappearance of parasites from the circulating blood. However, the tissues of lizard No. 1 (*C. collaris*) were examined after parasites had disappeared from the blood stream and no parasites were found.

The general distribution of parasites in *P. cornutum* differed from that described for *C. collaris*, in that endothelial cells were less frequently parasitized. Parasites occurred in greatest numbers in the spleen, and there was a decidedly greater density of parasites than in the circulating blood. The liver and bone marrow contained as many parasites as

the most vascular areas of the lungs, kidneys, and lamina propria of the intestine. Parasites were seen in all of the various types of cells observed infected in *C. collaris*, and in addition in plasma cells (Pl. 1, fig. 24) and binucleate reticular cells (Pl. 1, fig. 25) in the spleen.

Although the maximum number of parasites in the circulating blood of the most heavily infected specimens of *S. olivaceous* was over 20 times as great as that observed in *C. collaris* and *P. cornutum*, there were few exoerythrocytic stages in the circulating blood or in the tissues. The proportion of exoerythrocytic stages in the tissues of *S. olivaceous* did not at any time approach that attained in *C. collaris* and *P. cornutum*. The distribution of tissue stages in *S. olivaceous* also differed from that of the latter hosts, in that the bone marrow contained few parasites. The order of frequency with which parasites were encountered in the various tissues was spleen, liver, and endothelial cells of the heart and capillaries of the more vascular tissues. In the spleen, parasites occurred in macrophages, reticular cells, littoral cells, and hemocytoblasts. Littoral cells and macrophages of the liver were infected. In the heart, the parasites occurred in the endothelial cells and in the loose connective tissue of the sub-endothelial layer. The fixed cells of the bone marrow were not found to be infected. The chief activity in the bone marrow of *S. olivaceous* seemed to be that of granulopoiesis, and granulocytes

#### EXPLANATION OF PLATE

Plate 2.—Tissue stages of *Plasmodium mexicanum* in *Crotaphytus collaris*.

From sections of tissues fixed in Zenker-formol and stained by Maximow's hematoxylin eosin-azur method. Magnification  $\times 1500$ . Drawn by Miss Esther Bohlman.

1, numerous parasites in walls of small hepatic venule of the portal system. 2, capillary in the inner membrane covering the brain showing parasites in endothelial cells. 3, parasites in reticular cells of endosteum. 4, parasites in endothelial cells lining the intertrabecular spaces of the heart.

ac, cytoplasm of adventitial cell containing extravascular parasites; hn, nucleus of endothelial cell; s, segmenter; sch, schizont; t, trophozoite; p, parasite.



were rarely parasitized in this host.

There were relatively few exoerythrocytic stages in *S. undulatus*. Due to the presence of coccidia-like parasites in the tissues of most of these lizards, it is possible to describe the distribution of *P. mexicanum* in only one *S. undulatus*. In this animal all of the exoerythrocytic stages observed were of the *elongatum* type in that they occurred in circulating cells and no parasites were observed in such fixed cells as reticular, littoral, and true endothelial cells. In recapitulation, *P. mexicanum*, in a large degree, is a parasite of the fixed connective tissue cells in *C. collaris*, *Phrynosoma cornutum* and *P. asio*, but is mainly a parasite of the circulating blood cells in *S. olivaceus* and *S. undulatus*.

*The parasites.*—In general, the parasites in corresponding types of cells are similar in morphology and staining reaction in the various species of experimental hosts. An exception to this, however, occurs in the erythrocytic parasites in *P. cornutum*, which rarely form large schizonts such as typically occur in *S. olivaceous* and *S. undulatus*. Since only small numbers of gametocytes have been seen in *P. cornutum* and *P. asio*, it is not possible to make accurate comparison of these stages with those in other hosts, but no conspicuous differences have been detected in those encountered. This is also true of the asexual stages in the erythrocytes of *P. asio*. Since *Plasmodium mexicanum* has an asynchronous asexual reproduction, it is necessary to study a large number of parasites in order to determine whether there are size differences. This has not been possible in *Phrynosoma asio* because there were small numbers of circulating parasites in these infections. Similar to the situation in *Plasmodium elongatum* (Huff and Bloom, 1935) the segmenters in all hosts formed elongate merozoites,

and malarial pigment was produced only when the parasites occurred in cells possessing hemoglobin.

*P. mexicanum* is also remarkably similar to *P. elongatum* (Huff and Bloom, 1935) of birds in its occurrence in various types of blood cells. The degree of similarity in the distribution of parasites in different types of cells varies with the species of host. The distribution in *Phrynosoma cornutum* more closely resembles that of *Plasmodium elongatum* in canaries than does the distribution in any of the other hosts. In addition to the similarity between *P. mexicanum* and *P. elongatum*, the occurrence of exoerythrocytic stages of *P. mexicanum* in endothelial cells and macrophages parallels the occurrence of such stages of *P. cathemerium*, *P. relictum*, *P. circumflexum*, and *P. gallinaceum*—the so-called *gallinaceum*-type of exoerythrocytic stages (Porter, 1942).

Exoerythrocytic stages of *P. mexicanum* are also similar to those of both the *elongatum*- and *gallinaceum*-types in morphology and staining reactions. When stained by the Maximow technic trophozoites corresponding to the *elongatum* type are essentially like those described and represented by Huff and Bloom (1935). The parasite lies in a vacuole, the cytoplasm stains blue, and the nuclear material appears as a pale area in which there is a blue or purplish dot (Pl. 1, figs. 31–33). (The identity of the pale area and the dot in related parasites subjected to similar technics has been considered by Huff (1942). He compared wet and dry-fixed preparations of *P. elongatum*, *P. cathemerium*, and *Leucocytozoon simondi* and concluded that the pale area represents the nucleus and the dot represents the nucleolus.) The segmenters also are similar to exoerythrocytic segmenters of *P. gallinaceum* (Pl. 1, fig. 23, and Pl. 2, fig. 2) in the formation of elongate

merozoites, and in the fact that many more merozoites are produced per schizont than in the erythrocytic stages. The average number of erythrocytic segmenters is approximately 19, while many of the exoerythrocytic segmenters produce 72 or more merozoites.

When *P. mexicanum* is compared with the other species of *Plasmodium* in saurian hosts, it is found to resemble *P. maculilabre*. This parasite was described from several naturally infected lizards of the species *Mabuia maculilabris* (family Scincidae) in the Belgian Congo (Schwetz, 1931). Many of the figures and descriptions of the various stages of this parasite resemble corresponding stages of *P. mexicanum*. Although *P. maculilabre* was not described in great detail, the following differences are apparent and seem to be sufficient for distinguishing these parasites as distinct species. (1) Regardless of the position in the host cell, gametocytes of *P. maculilabre* were most commonly round and were rarely elongate, while those of *P. mexicanum* are usually elongate or elliptical and are round only when they occupy a polar position in the host cell. (2) *P. mexicanum* possesses the ability to parasitize a wide variety of cells in the different experimental hosts, and in the natural host frequently occurs in normoblasts (and possibly in other types of cells), while *P. maculilabre* apparently occurs only in erythrocytes. (3) *P. maculilabre* is a parasite of the family Scincidae, while *P. mexicanum* occurs in the family Iguanidae and attempts to establish it in 3 species of the family Scincidae were unsuccessful (table 1). (4) The 2 strains occurred in widely separated localities.

The species description of *P. mexicanum* is made from parasites as they appear in *S. olivaceous*. This host has been selected because the infection in the natural host consisted of few para-

sites (which were mainly gametocytes) and was not known to consist of a single species of parasite. Of the various experimental hosts, *S. olivaceous* seemed to be the most desirable from which to describe the parasite because: (1) it acquires heavy blood infections and supports extensive gametocyte development, (2) the majority of the parasites are in the red cell series, which apparently is true in the natural host, and (3) it is most like the natural host not only in belonging to the same genus (*Sceloporus*), but in general appearance. Drawings from various species of hosts are used to illustrate the morphology of *P. mexicanum* (Pl. 1, figs. 1-34). As was pointed out earlier, the parasites in corresponding types of cells in the different hosts are highly similar, with the possible exception of slightly smaller erythrocytic schizonts in *Phrynosoma cornutum* which are not included in Pl. 1. The following description has been made from blood smears stained with Giemsa's stain.

*Plasmodium mexicanum*, N. Sp.

*Trophozoites*.—(1) When in erythrocytes, normoblasts, and polychromatophil erythroblasts, these stages occur in various parts of host cell cytoplasm, cause no distortion or hypertrophy of host cell, are elongate or oval with irregular edges, have faint blue cytoplasm and deep rose chromatin, and the pigment consists of very fine, dark brown or black granules, or of an amorphous mass. (2) When in basophil erythroblasts, lymphocytes, monocytes, thrombocytes, stem cells, and granulocytes, the trophozoites are round or oval, have deep rose chromatin and light blue cytoplasm which frequently is difficult to distinguish from that of the host cell. The chromatin divides early and apparently only asexual stages develop in these cells. They cause much distortion of the host cell nucleus, except in polymorphonuclear granulocytes, and do not produce pigment.

*Schizonts and segmenters*.—(1) When in erythrocytes, normoblasts and polychromatophil erythroblasts, these stages are round or elongate, occupy various parts of the cytoplasm of the host cell, do not cause hypertrophy of the host cell but do cause nuclear displacement, have pigment



commonly consisting of a brown amorphous mass, and have deeply staining chromatin with the individual masses varying much in size and frequently very irregular in shape or dust-like. Segmenters form commonly 17 to 20 merozoites, which in dry smears may be round but are usually elongate. (2) When in basophil erythroblasts, lymphocytes, monocytes, thrombocytes, stem cells, and granulocytes, schizonts are round or oval. Merozoites are usually elongate and the number of merozoites varies with the size of the host cell—10 to 40 or more.

*Gametocytes.*—These occur only in cells that possess hemoglobin, occur frequently in normoblasts, cause much hypertrophy and distortion of the host cell, usually push the host nucleus to one side or less frequently toward one end of the cell, and occasionally partially surround the host nucleus. They are elliptical when alongside the host nucleus, and oval when in a polar position. They are approximately 12 to 16 $\mu$  long and 6 to 7.7 $\mu$  wide and have pigment consisting of 15 to 30 or more coarse, round or bacilloid, black granules, which are distributed irregularly in the cytoplasm. In macrogametocytes the cytoplasm stains deep blue, the nucleus is moderately discrete, and the nucleolus is conspicuous, usually appearing at the edge of the nucleus of the parasite as a purplish-red body approximately  $2.25 \times 1.5\mu$ . In old gametocytes the cytoplasm becomes vacuolate. In microgametocytes the cytoplasm stains light blue or pink and the nucleus is diffuse.

*Host cells.*—These are mainly erythrocytes and normoblasts, but small numbers of parasites occur in practically all types of circulating cells, including polychromatophilic and basophilic erythroblasts, lymphocytes, monocytes, thrombocytes, stem cells, and granulocytes.

*Experimental hosts:* *Sceloporus olivaceus*, *Sceloporus undulatus*, *Crotaphytus collaris*, *Phrynosoma cornutum*, *P. asio*.

*Sporogonous cycle and vectors:* Unknown.

*Type locality:* Michoacan, Mexico.

*Type host:* *Sceloporus ferrariperezi*.

## DISCUSSION

The description of exoerythrocytic stages of *P. mexicanum* constitutes the first record of such forms in a saurian host. This is possibly due to the fact that the tissues of very few infected lizards have been examined. The significance of this discovery is that, contrary to the popular belief, exoerythrocytic

stages are not confined to the malarial parasites of birds. The evidence that saurian, exoerythrocytic parasites represent stages in the life cycle of *Plasmodium* is, in general, similar to that cited for avian exoerythrocytic stages by Porter and Huff (1940), and the occurrence of these forms in both saurian and avian malaria lends support to the belief that these parasites in each instance are stages of *Plasmodium*. The exoerythrocytic stages of *P. mexicanum* occur in a wider variety of cells than those of any known species of avian malarial parasite. The stages in the lizard include the *elongatum* type of avian exoerythrocytic parasites, which occur in a great variety of blood and blood-forming cells, and those of the *gallinaceum* type which occur in macrophages and true endothelial cells. Apparently all types of cell parasitized by *P. elongatum* and by the species of avian parasites exhibiting the *gallinaceum* type of exoerythrocytic schizogony are also parasitized by *P. mexicanum*. Not only is there close parallelism in the types of cells parasitized in the two groups of hosts, but, as was pointed out earlier, exoerythrocytic stages in lizards closely resemble those of birds in morphology and staining reactions.

A comparison of saurian and avian malarial parasites indicates that, in addition to the occurrence of exoerythrocytic stages in both, there is in the erythrocytic parasites a high degree of morphological parallelism between various species of parasites in the two groups. This is evident when certain species of saurian and avian parasites are compared. For instance, it has been pointed out that *P. minasense* of the iguana resembles *P. tenue* of the Japan bird (Laveran and Marullaz, 1914) in size, development, and appearance (Wenyon, 1915). *P. minasense* and *P. rhadinurum* (see following paper) of

lizards are similar in size to *P. rouxi* and *P. vaughani* (probably the same as *P. tenue*). However, *P. rhadinurum* has only round gametocytes while each of the other species has elongate gametocytes, and it is this feature that makes it unique among the small plasmodia of lizards and birds. *P. stenocerci*, *P. tropiduri*, and *P. floridense* in the iguanid lizards (see following paper) constitute a group of parasites whose round gametocytes and schizonts are morphologically similar to those of *P. cathemerium* and *P. relictum* of passerine birds. Other examples of morphological parallelism are illustrated by *P. diploglossi* of lizards and *P. circumflexum* of birds, which have larger asexual and sexual forms that encircle the host cell nucleus; *P. agamae* of lizards and *P. hexamerium* of birds, which have elongate gametocytes and commonly produce 6 merozoites; and *P. giganteum* of lizards and *P. gallinaceum* of birds, whose general morphology and average number of merozoites are similar.

The occurrence of exoerythrocytic stages in both groups of *Plasmodium* and the morphological parallelism between different species in the 2 groups suggest that the malarial parasites in lizards and birds have evolved from a common ancestral stock. While there are several hypotheses that may be proposed to describe the evolution of these parasites, it seems most probable that such an ancestral stock existed in the prereptilian ancestors and had already begun to give rise to different species of parasites by the time birds and lizards evolved as distinct classes of animals. It would seem likely also that such a primitive stock may have existed in a very wide variety of cells and as different species of *Plasmodium* were developed, morphological changes occurred and the ability to parasitize various types of cells was lost

in different ways. Thus, of the known species of parasites, *P. mexicanum* appears to have remained the most like such a hypothetical primitive form. According to this hypothesis *P. elongatum* may have lost the ability to infect endothelial cells, and *P. cathemerium*, *P. relictum*, *P. circumflexum*, and *P. gallinaceum* may have lost the ability to infect many of the cell types now infected by *P. elongatum*. This hypothesis does not attempt to answer the question of whether malarial parasites first existed in vertebrates or invertebrates, but it agrees best with the belief of Huff (1938) that invertebrate hosts were antecedent to the vertebrate hosts.

The data on the comparative behavior of *P. mexicanum* in different species of lizards illustrate the effects of the species of the host upon the parasite and the nature of the infection. The variations in the course of the infection, production of gametocytes, and cellular distribution of parasites in different species of lizards are due to differences in the hosts rather than to alterations in the parasites (as, for example, *Dauermodifikationen*). This is indicated by the fact that the parasites exhibited a characteristic behavior in each species of host although all of the lizards of each species were not infected in sequence. In general, the effects of the species of the host are comparable to those of the species of the host in simian and avian malaria. Differences were observed in the morphology and course of infection of *P. brasilianum* in several species of Panamanian monkeys by Taliaferro and Taliaferro (1934). In avian malaria, *P. relictum* produced elongate gametocytes when transferred to the duck, *Anas boschas domestica* (Wolfson, 1939). The species of the host has been found to influence the occurrence of exoerythrocytic schizogony in avian malaria by Rodhain (1938a; 1938b) and Wolfson



(1940). In *P. mexicanum*, the effects of the species of the host on the cellular distribution of the parasites were very marked. Although the types of cells parasitized has been suggested as one of the criteria useful in classifying avian plasmodia (Manwell, 1936), the variations in this respect illustrate the danger of relying upon the type of host cell as a criterion for determining the species of malarial parasites in lizards. The effect of the species of the host on gametocyte development was demonstrated when *P. falciparum* was transferred to howler monkeys (*Alouatta sp.*) and failed to produce gametocytes, although in several of these animals asexual stages became relatively numerous (Taliaferro and Taliaferro, 1934b). The failure of *P. mexicanum* to produce gametocytes in collared lizards is analogous to this failure of *P. falciparum* to produce gametocytes in the howler monkey, the most conspicuous difference between the two cases being that *P. mexicanum* became largely an exoerythrocytic parasite when transferred to *C. collaris*—a transition possibly associated with the absence of gametocytes. Opposed to this explanation is, however, the observation that gametocytes of *P. mexicanum* were produced in *Phrynosoma asio* although over 99% of the parasites were exoerythrocytic. An alternative hypothesis for the absence of gametocytes in *C. collaris* may be that the physio-chemical complex of the blood and tissues of this host differs too greatly from that of the natural host, *S. ferrariperezi*, and from *S. olivaceous* and *S. undulatus*, to allow the maturation of the gametocytes. According to this hypothesis, it would follow that *Phrynosoma cornutum* and *P. asio* differ less in this respect from the natural host than does *C. collaris*. The degree of kinship existing among the 3 genera of lizards is unknown, but the data on

gametocyte production suggest that *Phrynosoma* is more closely related to *Sceloporus* than is *Crotaphytus*. Precipitin tests on the various hosts might be of value in indicating the significance of differences in parasite behavior as a criterion for determining the degree of kinship among hosts of different species.

The occurrence of gametocyteless infections in *C. collaris* and of few gametocytes in *Phrynosoma* was clearly a function of the species of host and was not due to a permanent change in the parasite. However, in avian malaria Gambrell (1937) has observed a gametocyteless strain which resulted from an apparent inherent change in the parasite. She found that a strain of *P. cathe-merium* which had become gametocyteless while being maintained in canaries by blood inoculation also failed to produce gametocytes in the natural hosts, English sparrows.

The extensive occurrence of exoerythrocytic stages of *P. mexicanum* is a characteristic probably unfavorable to the survival of the species. In the malaria of lizards, evidence for this statement is found in the general inverse relationship between the proportion of parasites of the exoerythrocytic type and the proportion of gametocytes. Apparently gametocytes develop only in cells that possess hemoglobin. If further study confirms this, it seems that the postulated transition of *Plasmodium* from a parasite of many cell types to an erythrocytic parasite may be regarded as an adaptive change since larger numbers of gametocytes would be produced, thereby increasing the likelihood of successful arthropod transmission and survival of the species.

#### SUMMARY AND CONCLUSIONS

*P. mexicanum*, n. sp., was isolated from a lizard of the species *Sceloporus ferrariperezi* obtained from Michoacan,

Mexico, by inoculating blood into the lizard *Crotaphytus collaris*. This parasite has been established also in *Phrynosoma cornutum*, *Phrynosoma asio*, *Sceloporus undulatus*, and *S. olivaceus*. Study of the infections in each of these hosts has made possible the following observations.

- (1) *P. mexicanum* produces exoerythrocytic stages in each species of host that correspond to the two types of avian exoerythrocytic stages. These are the *elongatum* type, which occur in a great variety of blood and blood-forming cells, and the *gallinaceum* type, which occur in macrophages and true endothelial cells.
- (2) The general distribution of the parasites is described in *C. collaris*, *Phrynosoma cornutum*, and *S. olivaceus*. In the first 2 species of hosts, they are largely parasites of fixed connective tissue cells, including reticular cells, endothelial cells of capillaries, littoral cells of the liver, and macrophages. The various blood and blood-forming cells are also parasitized commonly. In *S. oli-*

*vaceus* they are largely parasites of circulating blood cells. While the same types of fixed cells are parasitized as in *C. collaris*, the number of parasites in these cells is very much less.

- (3) The species of the host also influences: (a) the distribution of parasites in the different types of circulating blood cells and the proportion of the parasite population represented by exoerythrocytic stages; (b) whether or not gametocytes are produced; and (c) the course of the infection.
- (4) *P. mexicanum* has an asynchronous asexual reproduction.

Attention is called to the striking parallelism existing between the species of *Plasmodium* in birds and similar forms in lizards. The occurrence of exoerythrocytic stages in the malarial parasites of both groups suggests that these parasites may have evolved from a common ancestral stock, perhaps, similar in its cell preferences to *P. mexicanum*.

#### REFERENCES

(On pp. 78-79 of the following article.)



# SAURIAN MALARIAL PARASITES OF THE UNITED STATES AND MEXICO

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In the preceding article (pp. 48-67) a new species of saurian malarial parasite, *Plasmodium mexicanum*, capable of living in all blood and blood-forming cells and also in macrophages and the endothelium of capillaries was reported. The discovery of this parasite resulted from a survey of the blood parasites of North and Central American lizards, the full results of which are recorded in the account following. Two more new species are described and the data from experiments involving them are reported. A short account is also included of certain unidentified parasites and bodies of an obscure nature which were encountered during the course of these experiments and those of the preceding paper.

## MATERIALS AND METHODS

*The strains of parasites.*—Three strains representing different species of saurian malarial organisms have been isolated and studied experimentally and, in addition, smears from a large number of naturally infected lizards have been examined. The sources of these are indicated in the section on the natural incidence of blood parasites. The strains studied include *Plasmodium mexicanum*, n. sp. (described in the preceding article, pp. 48-67) in *Sceloporus ferrariperezi* obtained from Michoacan, Mexico; *Plasmodium rhadinurum*, n. sp. in *Iguana iguana rhinolopha* from Colima, Mexico; *Plasmodium floridense*, n. sp., in *Scelo-*

*porus undulatus* obtained from Ocala, Florida.

*The lizards and their care.*—The species of lizards studied are described in the section on natural incidence and in the Materials and Methods section of the preceding paper (pp. 48-49). The methods for maintaining the lizards alive in the laboratory are likewise discussed there.

*Technics for bleeding and inoculating the lizards and for the staining of smears and sections.*—See p. 49 of the preceding paper.

## OBSERVATIONS ON *Plasmodium* *rhadinurum*, N. SP.

This interesting parasite was discovered in a green iguana (*Iguana iguana rhinolopha*) procured from Colima, Mexico. It occurs in erythrocytes, forms pigment and undergoes schizogony in erythrocytes. It is unique among the known plasmodia, however, in that many of the trophozoites in each smear have long slender cytoplasmic processes (see preceding paper, Pl. 1, figs. 39-43), a characteristic that is retained when the parasites are established in a different species of host.

The natural host was kept in the laboratory for 6 weeks, during which time the parasites were studied and blood inoculations were made into other species of lizards. Infections were established in 2 juvenile lizards (*Sceloporus undulatus*), while one lizard of each of the following species was inoculated intraperitoneally with negative results: *Ctenosaura acanthura*, *Crotaphytus collaris*, *Sceloporus olivaceus*,

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*Phrynosoma cornutum*, *Eumeces laticeps*, and *E. obsoletus*. Blood smears from the natural host and from experimentally infected lizards constituted the material from which this parasite was studied.

*The infection in Iguana i. rhinolopha.*—The infection in the naturally infected green iguana remained at a low level (about 190 parasites per 10,000 erythrocytes) during the entire 6 weeks of observation, with no obvious change in the number of parasites. All of the parasites were in erythrocytes, and the infection was asynchronous, with parasites in all stages of development occurring in each smear. Although a few gametocytes of both sexes were seen, these constituted a very small proportion of the parasite population. Twenty-five per cent of all parasites had definite cytoplasmic processes.

*The infections in S. undulatus.*—Following intraperitoneal inoculations, the 2 infections that resulted were light, the maximum number of parasites being 83 per 10,000 blood cells. The 2 lizards inoculated received 0.1 cc and 0.05 cc of blood, respectively. The incubation periods were 7 and 12 days. The shorter incubation period occurred in the lizard receiving the larger inoculum. It continued to be infected during the next 14 days, and then died. Twenty-four per cent of the parasites possessed one, or less frequently, 2 cytoplasmic processes. In all respects the parasites had the same morphology and staining reactions in *S. undulatus* as in *I. rhinolopha*.

Parasites continued to appear in the lizard receiving the smaller inoculum until it was killed 6 days after parasites first appeared. At this time the number of parasites was decreasing and the host was killed for tissue studies. The tissues were sectioned and examined unsuccessfully for exoerythrocytic stages, but the infection was at a very low level when the lizard was killed. All parasites ob-

served in *S. undulatus* were in erythrocytes.

*Relationship to Plasmodium minasense.*—*P. rhadinurum* is similar to *P. minasense* which has been described in the lizard *Mabuia agilis* from Minas Geraes, Brazil, by Carini and Rudolph (1912) and in *Iguana sapidissima* from Trinidad by Wenyon (1915). The description by Carini and Rudolph is inadequate for determining whether these workers observed *P. rhadinurum*, but there are 3 distinct differences between *P. rhadinurum* and *P. minasense* as described by Wenyon. *P. minasense* has both round and halteridium-like gametocytes, produces only 4 merozoites, and does not have long cytoplasmic processes. *P. rhadinurum* has only round gametocytes, occasionally produces 5 merozoites, and about one-fourth of the asexual parasites have long cytoplasmic processes. Blood smears containing *P. rhadinurum* have been sent recently to Dr. Wenyon for comparison with smears containing *P. minasense*, and he has failed to find these cytoplasmic processes upon reexamining the latter species. In spite of the similarity in other respects these morphological differences seem to be adequate for the designation of this parasite as a separate species, and it is proposed that it be called *Plasmodium rhadinurum*, n. sp. The name "rhadinurum," which means a slender-tailed organism, was suggested by Professor Gertrude Smith of the Department of Greek of the University of Chicago. Drawings of various stages of the parasite stained with Giemsa's stain are shown in Plate 1, figs. 35-44 (see preceding paper).

*Plasmodium rhadinurum*, n. sp.

*Trophozoites.*—These stages occur in various parts of the host cell cytoplasm, but usually occupy a polar position. They do not cause distortion or hypertrophy of host cells. The shape of the trophozoite varies extensively: some are thin



and elongate with the cytoplasm assuming a great variety of shapes, others are round or oval and compact, and a large proportion have 1 or 2 long cytoplasmic processes, which are the only parts commonly touching the host nucleus. The pigment is scanty, consisting of a few fine, dark granules, the cytoplasm is vacuolated and the nucleus stains deeply, giving the entire parasite a reddish appearance. *Schizonts and segmenters*.—These stages usually are at one end of the host cell, do not cause distortion or hypertrophy of the cell, and are small, being approximately  $4\mu$  in diameter. Schizonts are more compact than trophozoites, and may be round, elongate, or irregular. The parasite usually appears pink, probably due to the relatively large size of the nuclei and the small amount of cytoplasm. Segmenters form 4, or occasionally, 5 merozoites, which may be arranged in a cross or fan-shaped configuration. *Gametocytes*.—These forms occupy a polar position in the host cell, displace the host nucleus (although causing very little hypertrophy of the host cell) and are round and much larger than the asexual forms, ranging from  $6.5$  to  $7.7\mu$  in diameter. In macrogametocytes the nucleus is moderately discrete and stains light red, the cytoplasm stains medium blue, and the pigment consists of 35 or more fine, black granules which are more nearly round than bacilloid, and are distributed irregularly in the cytoplasm of the parasite. In microgametocytes the nucleus is diffuse and stains pink, the cytoplasm stains lightly, and the pigment granules, although apparently fewer in number, are similar to those in macrogametocytes.

*Host cells*: Erythrocytes.

*Sporogonous cycle and vectors*: Unknown.

*Experimental hosts*: *Sceloporus undulatus*.

*Type host*: *Iguana iguana rhinolopha*.

*Type locality*: Colima, Mexico.

#### *Malarial Parasites in Florida Lizards*

The presence of *Plasmodium* in lizards from Florida was reported by Huff (1941). Subsequent studies have revealed numerous infections in *Sceloporus undulatus* and *Anolis carolinensis* obtained from the vicinity of Ocala, Florida. (The species of lizards and the numbers examined are given in table 5, in the section on the natural incidence of *Plasmodium* in saurian hosts.) In each infection the parasites exhibited considerable variation in the shape of

the gametocytes and in the number of merozoites produced by a segmenter. In each instance both elongate and round gametocytes occurred and the position of these stages in the host cell seemed to influence the shape of the parasite. When the gametocytes were along the side of the host cell nucleus they usually were elongate, but if they occurred in a polar position in the host cell they were commonly round or ovoid. In poorly fixed host cells these stages were round or ovoid, and as the gametocytes approached maturity there was less tendency to assume an elongate shape. While it is conceivable that these infections consisted of 2 species of parasites, the constant occurrence of these characteristics in every heavily infected lizard seems to warrant the conclusion that a single species is involved. This parasite has not been described before and it is proposed that it be called *Plasmodium floridense*, n. sp.

A comparison of *P. floridense* with the previously described species of *Plasmodium* in saurian hosts reveals that it resembles *P. tropiduri* and *P. stenocerci*. *P. tropiduri* was described from *Tropidurus torquatus* (Aragão and Neiva, 1909) while *P. stenocerci* was described from *Stenocercus* sp. (Carini, 1941). It was pointed out by Carini (1941) that *P. stenocerci* is very similar to *P. tropiduri* and that these may later prove to be the same species, but since they differed in certain respects and were found in different hosts, he described *P. stenocerci* as a new species. *P. floridense* is similar to these parasites in certain respects, but it differs from *P. tropiduri* and *P. stenocerci* morphologically more than the latter 2 species differ from each other. In addition, it occurs in different species of hosts and in a locality hundreds of miles from those in which the other species occur. The 3 species form a closely related

group. Differences among them are shown in table 1.

The material from which *P. floridense*, n. sp. is described consists of air-dried blood smears from a naturally infected *Sceloporus undulatus*. The smears were stained with MacNeal's tetrachrome stain. Representative stages of this species are shown in Pl. 1, figs. 45-54 of the preceding article.

polar position the shape varies from round to ovoid with the side next to the host nucleus indented. Immature elongate pregametocytes frequently have an irregular border on the side adjacent to the host nucleus, and pigment forms at the extremities of the parasite. Mature forms have a regular outline, and are round (7.5-8.0 $\mu$  in diameter), ovoid, or elongate but with a less pronounced elongate shape than that of the immature forms. Macrogametocytes have moderately blue cytoplasm, frequently the nucleolus is visible, and the pigment consists of 30 or more fine,

TABLE 1.—Comparison of Characters of *P. tropiduri*, *P. stenocerci*, and *P. floridense*, n. sp.

|                                         | <i>P. tropiduri</i>              | <i>P. stenocerci</i>            | <i>P. floridense</i> , n. sp.                 |
|-----------------------------------------|----------------------------------|---------------------------------|-----------------------------------------------|
| Position in host cell                   | Polar                            | Polar                           | Various positions                             |
| Effects upon host cell                  | Hypertrophy<br>Nucleus displaced | No hypertrophy<br>No distortion | Little or no hypertrophy<br>Nucleus displaced |
| Shape of gametocytes                    | Round                            | Round                           | Round, ovoid or elongate                      |
| Shape of schizonts                      | Round or oval<br>7-8 in diameter | Round<br>5-6 in diameter        | Elongate, round or irregular                  |
| Number of merozoites                    | Maximum 12                       | 10-12                           | 6-21; average 12                              |
| Relative frequency, segmenters in blood | Not mentioned                    | Rare                            | Common                                        |
| Multiple infections of host cells       | Common                           | Not mentioned                   | Common                                        |
| Type host                               | <i>Tropidurus torquatus</i>      | <i>Stenocercus</i> sp.          | <i>Sceloporus undulatus</i>                   |
| Type locality                           | State of Minas, Geraes, Brazil   | State of Goyaz, Brazil          | Ocala, Florida                                |

#### *Plasmodium floridense*, n. sp.

Two or more parasites frequently occur in the same host cell. The asexual stages occur in various parts of the host cell cytoplasm and do not cause hypertrophy or distortion of the cell. *Trophozoites*.—The smaller forms are elongate or pyriform, have light blue cytoplasm and pink chromatin which may occur in any part of the parasite, and do not have pigment. Medium-sized trophozoites are elongate, pyriform, or round, and the pigment consists of several discrete, dark brown or black granules, which may be distributed irregularly through the cytoplasm or grouped in a loose aggregation around a particular locus. *Schizonts and segmenters*.—These stages vary from round or elongate shape (sausage-shape) to irregular hour-glass shape (with the pigment in the slender portion). They are usually elongate when at the side of the host nucleus, and in this position may bend around the end of the host nucleus or occasionally extend the length of the cell. The pigment consists of an aggregation of golden brown or dark brown granules. Segmenters form 6 to 21 or more merozoites, the average being approximately 12. The merozoites frequently are elongate. *Gametocytes*.—The sexual stages occurring in various parts of the host cell cause very little hypertrophy but considerable distortion of the host cell. They vary in shape depending upon the position in the host cell. When along the side of the host nucleus they are elongate, but when in a

round (or nearly round) granules widely distributed in the cytoplasm of the parasite. Microgametocytes have a diffuse nucleus, light blue or pink cytoplasm, and the pigment consists of approximately 20 to 30 fine, irregularly shaped, black or dark brown granules.

*Host cells*: Mainly erythrocytes and very rarely erythroblasts.

*Sporogonous cycle and vectors*: Unknown.

*Type host*: *Sceloporus undulatus*.

*Type locality*: Vicinity of Ocala, Florida.

*P. floridense* has been transferred to *C. collaris*, *C. wislizenii*, and *A. carolinensis* by inoculating with blood from an infected *S. undulatus*. *Cnemidophorus tessellatus*, *Phrynosoma cornutum*, and *Phrynosoma orbiculare* were refractory to infection (Huff, 1941). Tissue studies of natural and experimentally-induced infections failed to reveal exoerythrocytic stages (Huff, 1941). We have transferred this parasite by intraperitoneal inoculation of blood from a naturally infected *S. undulatus* to uninfected lizards of the same species obtained from North Carolina. The incubation periods of the infections in 3 of these hosts were 10, 17, and 39 days, respec-



tively. The infections persisted until the lizards were killed or died, 10 to 31 days after parasites first appeared in the blood, but all of the infections were relatively light. Gametocytes appeared in each lizard, and in one infection were observed to represent 17% of the parasite population. The asexual reproduction of the parasites was studied in one of the lizards. The animal was kept in

#### THE NATURAL INCIDENCE OF BLOOD PARASITES IN NORTH AND CENTRAL AMERICAN LIZARDS

During the past 2 years we have examined a considerable number of lizards for blood parasites. Grateful appreciation is extended to the following people who have supplied a considerable portion of the lizards examined: Mr. Harry Hoogstraal of the University



Fig. 1.—Asexual reproduction of *P. floridense* in an experimentally infected *S. undulatus* as indicated by the percentage of segmenters and schizonts with more than 4 nuclei for a period of 5 days.

the light from 6 A.M. to 7 P.M. and in the dark from 7 P.M. to 6 A.M. The environmental temperature fluctuated with the diurnal rhythm characteristic of late summer. Blood smears were made at 6-hour intervals for a period of 5 days (from the 7th through the 11th day after parasites first appeared in the blood). The number of parasites having 5 or more nuclei, or undergoing segmentation, was determined for 50 parasites in each smear. (Segmenters in this strain commonly produce 6 to 21 merozoites.) The percentage of segmenters and mature schizonts in each smear is given in fig. 1. The infection is asynchronous, with the number of segmenters ranging between 2 and 10%.

of Illinois; Mr. T. H. Frison of the Illinois Natural History Survey Division; Mr. Fred R. Cagle of Southern Illinois Normal University; and Mrs. Elfreda L. Caldwell, Mary Esther, Florida. The writer also wishes to thank Mr. Karl P. Schmidt and Mr. Clifford H. Pope of the Chicago Museum of Natural History for valuable assistance in identifying the lizards. The study includes a total of 1,136 lizards, belonging to 37 species and representing 6 families. The lizards have been obtained from Michoacan, San Luis Potosi, and Colima in Mexico, and from Arizona, Florida, California, Illinois, Louisiana, North Carolina, Texas, and Utah. The lizards recorded as coming

from Central Floridá were purchased from dealers, and the exact localities from which the specimens were collected zoa, 2.7%; and microfilariae, 1.1%. The species of lizards examined for blood parasites, the localities repre-

TABLE 2.—*Species of North and Central American Lizards Examined for Blood Parasites, the Localities Represented, and the Results of the Examinations*

| Hosts<br>Family and species           | Locality                       | Parasites               |                                         |                               |                    |                 |
|---------------------------------------|--------------------------------|-------------------------|-----------------------------------------|-------------------------------|--------------------|-----------------|
|                                       |                                | <i>Plasmo-<br/>dium</i> | Uniden-<br>tified<br>Haemo-<br>sporidia | Uniden-<br>tified<br>Sporozoa | Micro-<br>filariae | No.<br>Negative |
| Anguidae                              |                                |                         |                                         |                               |                    |                 |
| <i>Gerrhonotus</i> spp.               | Mexico, Michoacan              |                         |                                         |                               |                    | 20              |
| <i>Ophisaurus ventralis</i>           | Florida                        |                         |                                         |                               |                    | 2               |
| Geckonidae                            |                                |                         |                                         |                               |                    |                 |
| <i>Phyllodactylus</i> spp.            | Mexico, Michoacan              |                         |                                         |                               |                    | 3               |
| <i>Sphaerodactylus cinereus</i>       | Florida                        |                         |                                         |                               |                    | 2               |
| Helodermatidae                        |                                |                         |                                         |                               |                    |                 |
| <i>Heloderma horridum</i>             | Mexico                         |                         |                                         |                               |                    | 1               |
| Iguanidae                             |                                |                         |                                         |                               |                    |                 |
| <i>Anolis carolinensis</i>            | Florida, Ocala                 | 32                      | 5                                       |                               | 11                 | 20              |
| <i>Anolis carolinensis</i>            | Florida, Mary Esther           |                         |                                         |                               |                    | 3               |
| <i>Anolis carolinensis</i>            | Louisiana, Thibodeaux          |                         |                                         | 3                             |                    | 176             |
| <i>Anolis carolinensis</i>            | Louisiana, New Orleans         |                         |                                         |                               |                    | 20              |
| <i>Basiliscus vittatus</i>            | Mexico, Michoacan              | 1                       |                                         |                               |                    | 2               |
| <i>Crotaphytus collaris</i>           | Texas, Palo Pinto              |                         |                                         | 5                             |                    | 120             |
| <i>Crotaphytus wislizenii</i>         | Utah                           |                         |                                         |                               |                    | 1               |
| <i>Ctenosaura acanthura</i>           | Mexico, Colima                 |                         |                                         | 1                             | 1                  | 0               |
| <i>Iguana iguana rhinolopha</i>       | Mexico, Colima                 | 3                       |                                         |                               |                    | 2               |
| <i>Iguana iguana rhinolopha</i>       | Mexico, Michoacan              |                         |                                         |                               |                    | 2               |
| <i>Phrynosoma asio</i>                |                                |                         |                                         |                               |                    | 3               |
| <i>Phrynosoma coronatum</i>           | California                     |                         |                                         |                               |                    | 12              |
| <i>Phrynosoma cornutum</i>            | Texas                          |                         |                                         |                               |                    | 58              |
| <i>Phrynosoma</i> spp.                | Mexico                         |                         |                                         |                               |                    | 7               |
| <i>Sauromatus ater</i>                | Arizona                        |                         |                                         | 1                             |                    | 1               |
| <i>Sceloporus aeneus</i>              | Mexico, Michoacan              |                         |                                         |                               |                    | 19              |
| <i>Sceloporus ferrariiperezi</i>      | Mexico, Michoacan              | 23                      | 5                                       |                               |                    | 38              |
| <i>Sceloporus graciosus</i>           | Utah                           |                         |                                         |                               |                    | 1               |
| <i>Sceloporus horridus oligoporus</i> | Mexico, Michoacan              | 1                       |                                         |                               | 1                  | 7               |
| <i>Sceloporus microlepidotus</i>      | Mexico, Michoacan              | 1                       | 1                                       | 6                             |                    | 28              |
| <i>Sceloporus olivaceus</i>           | Texas, Palo Pinto              |                         |                                         |                               |                    | 37              |
| <i>Sceloporus pyrocephalus</i>        | Mexico, Michoacan              | 1                       |                                         |                               |                    | 13              |
| <i>Sceloporus scalaris</i>            | Mexico, Michoacan              |                         |                                         |                               |                    | 3               |
| <i>Sceloporus</i> spp.                |                                |                         |                                         |                               |                    | 4               |
| <i>Sceloporus undulatus</i>           | Florida, Ocala                 | 44                      | 3                                       | 8                             |                    | 135             |
| <i>Sceloporus undulatus</i>           | Florida, Mary Esther           |                         |                                         | 4                             |                    | 78              |
| <i>Sceloporus undulatus</i>           | North Carolina, Elon           |                         |                                         |                               |                    | 12              |
| <i>Sceloporus undulatus</i>           | Illinois, Mason and Carbondale |                         |                                         |                               |                    | 41              |
| <i>Sceloporus woodi</i>               | Florida, Englewood             |                         |                                         | 3                             |                    | 24              |
| <i>Sceloporus variabilis</i>          | Mexico, San Luis Potosi        | 1                       |                                         |                               |                    | 4               |
| Scincidae                             |                                |                         |                                         |                               |                    |                 |
| <i>Eumeces fasciatus</i>              | Illinois and ?                 |                         |                                         |                               |                    | 3               |
| <i>Eumeces obsoletus</i>              | Texas, Palo Pinto              |                         |                                         | 1                             |                    | 10              |
| <i>Eumeces laticeps</i>               | Illinois, Diona                |                         |                                         |                               |                    | 3               |
| Teiidae                               |                                |                         |                                         |                               |                    |                 |
| <i>Ameiva undulata</i>                | Mexico, Michoacan              |                         |                                         |                               |                    | 6               |
| <i>Cnemidophorus deppii</i>           | Mexico, Michoacan              |                         |                                         |                               |                    | 21              |
| <i>Cnemidophorus gularis</i>          | Mexico, Michoacan              |                         | 1                                       |                               |                    | 23              |
| <i>Cnemidophorus guttatus</i>         | Mexico, San Luis Potosi        |                         |                                         |                               |                    | 4               |
| <i>Cnemidophorus sexlineatus</i>      | Florida, Mary Esther           |                         |                                         |                               |                    | 1               |
| <i>Cnemidophorus</i> spp.             | Mexico and ?                   |                         |                                         |                               |                    | 12              |
| Totals                                |                                | 107                     | 15                                      | 32                            | 13                 | 982             |

could not in all cases be accurately determined. The incidence of blood parasites was determined by examining air-dried smears stained with tetrachrome or Giemsa's strain.

The percentages of lizards harboring various types of blood parasites were *Plasmodium*, 9.4%; unidentified Haemosporidia, 1.3%; unidentified sporo-

zoa, 2.7%; and microfilariae, 1.1%. The species of lizards examined for blood parasites, the localities repre-

sented, and the results of the examination are given in table 2. The data in table 2 seem to justify certain inferences regarding the differential occurrence of blood parasites in various families of lizards, and the geographical distribution of *Plasmodium* in saurian hosts. These inferences, along with the results of pertinent studies by



other workers, are presented in the discussion.

The majority of the malarial infections consisted of *P. mexicanum*, *P. floridense*, and *P. rhadinurum*. The possibility of the presence of other species is not excluded, however, since in many cases the infections were light and many of the smears were examined briefly. The infections listed in table 2

tissues of several of the lizards infected with microfilariae.

#### Possible Vectors

*P. floridense* was observed to form an oöcyst in *Aedes aegypti* (Huff, 1941). In the present study, 61 *Aedes aegypti* were allowed to feed upon *Sceloporus olivaceous* containing numerous *P. mexicanum* gametocytes. The mosquitoes

TABLE 3.—Results of Feeding *Aedes aegypti* and *Culex pipiens* on Lizards Infected with *P. rhadinurum* and on an Uninfected Lizard

| Experiment number | Lizard                               | Species of mosquito  | Number of mosquitoes | Results                 |
|-------------------|--------------------------------------|----------------------|----------------------|-------------------------|
| 1                 | Infected <i>Iguana i. rhinolopha</i> | <i>Aedes aegypti</i> | 100                  | All died within 24 hrs. |
| 2                 | Infected <i>Iguana i. rhinolopha</i> | <i>Aedes aegypti</i> | 33                   | All died within 24 hrs. |
| 3                 | Infected <i>S. undulatus</i> no. 1   | <i>Aedes aegypti</i> | 18                   | All died within 24 hrs. |
| 4                 | Infected <i>S. undulatus</i> no. 2   | <i>Culex pipiens</i> | 14                   | All died within 24 hrs. |
| 5                 | Infected <i>S. undulatus</i> no. 2   | <i>Culex pipiens</i> | 5                    | All moribund*           |
| 6                 | Uninfected <i>S. undulatus</i>       | <i>Culex pipiens</i> | 13                   | All lived               |

\* Unable to fly after 10 hours and killed for microscopic study.

as unidentified haemosporidia consisted of a very small number of pigmented parasites of which it was not possible to determine the genus.

The unidentified sporozoa included non-pigmented blood parasites. In most instances they were relatively large parasites of erythrocytes and resembled the gametocyte stage of *Haemogregarina*. The non-pigmented parasites in *A. carolinensis* and *C. collaris* were smaller than those resembling gametocytes of *Haemogregarina*, and they occurred in a variety of cells. These included granulocytes and lymphocytes of *A. carolinensis*, and erythrocytes and lymphocytes of *C. collaris*. The parasites of these hosts were similar to the sporozoites of certain haemococcidia.

Microfilariae were numerous in *A. carolinensis* from Florida. They did not have a sheath and were relatively broad in proportion to the length. The average dimensions of 10 fixed and stained specimens were  $59.5 \times 7.0 \mu$ . Adult filarial worms were found in the subcutaneous

were kept in incubators at 22 and 28 C and were dissected from 5 to 33 days after feeding, with no oöcysts or sporozoites being found.

*Aedes aegypti* and *Culex pipiens* were allowed to feed upon an *Iguana i. rhinolopha* and 2 specimens of *S. undulatus* infected with *P. rhadinurum*. For some unknown reason virtually all of these mosquitoes died within 24 hours after feeding, even though they were kept under the general conditions provided for the stock colonies of mosquitoes in the laboratory and different groups were kept at temperatures of 22 or 28 C. This observation became especially interesting when it was found that all of a small group of *Culex pipiens* lived after feeding on an uninfected *S. undulatus*. The number of mosquitoes fed on each lizard and the results of the feeding are given in table 3. These observations suggest that the parasites were lethal for the mosquitoes used.\*

\* Thanks are extended to Miss Helen Jordan for feeding and dissecting the mosquitoes.

UNIDENTIFIED PARASITES AND BODIES  
OF AN OBSCURE NATURE

Two specimens of an organism whose nature is obscure were seen in the blood of *Phrynosoma cornutum*. The organism was free in the plasma, and was  $60.2\mu$  long and  $1.4\mu$  in diameter in its broadest portion. The ends were pointed, and there was a dark staining area in the center which probably represented the nucleus (Pl. 1, fig. 56, preceding paper). This organism resembles *Sergentella hominis*, described in the blood of a man by Brumpt (1910), and a similar form described in the blood of a canary by Huff (1939).

In addition to the blood parasites, two other forms have been encountered in the tissues of various lizards. One of these resembles a coccidium, while the other more closely resembles the inclusion bodies associated with virus infections of the psittacosis type (Pl. 1, figs. 55 and 57). The identity and relationship of these forms have not been determined.

The coccidia-like parasites were observed in the tissues of one *S. undulatus* and in several specimens of *P. cornutum*. In each instance they were observed in lizards experimentally infected with *P. mexicanum*, which probably was coincidental, since most of the tissues studied came from experimentally infected animals. Since *P. mexicanum* in these hosts produces tissue parasites, it is not possible to accurately distinguish all stages of the malarial parasites in tissues from those of the coccidial type. Segmenters of the malarial parasites give rise to elongate merozoites which can be distinguished from the round merozoites of the coccidial type. In addition, the coccidia-like parasites occur commonly in hepatic cells while *P. mexicanum*, like other species of *Plasmodium*, has never been observed to

infect these cells. Both types of parasites, however, occur in the spleen.

The forms resembling inclusion bodies of certain viruses were observed in a specimen of *Phrynosoma asio* experimentally infected with *P. mexicanum*, and in the *S. undulatus* lizard infected with *Plasmodium mexicanum* and the coccidia-like parasites. These 3 forms are probably unrelated since *P. mexicanum* has been observed alone in numerous lizards, and bodies of the inclusion type have been observed alone in one species of *S. undulatus*. The discovery of forms resembling virus inclusion bodies in the lizard is especially interesting since viruses apparently have not been described in the Class Reptilia. These bodies have been found in hepatic cells, phagocytic cells of the spleen and bone marrow, fibroblasts in the loose connective tissue of the peritoneum, and in the lining cells of the lungs. In the lungs they are remarkably similar to the inclusion bodies of several virus infections including psittacosis and mouse pneumonitis (Dr. F. B. Gordon, Department of Bacteriology and Parasitology of the University of Chicago, 1943). In one *S. undulatus* that was not infected with *P. mexicanum* or the coccidia-like parasites, many multinucleate connective tissue cells in various stages of mitoses were observed in an area of the peritoneum containing the bodies of the inclusion type. These cells resembled the foreign-body, giant cells of higher vertebrates, but they became multinucleate as a result of nuclear division rather than through fusion, which is commonly believed to be the method by which giant cells develop in other animals. It seemed that some agent was stimulating mitosis in these cells, many of which contained as many as 6 distinct nuclei, but the available evidence does not indicate whether the inclusion-like bodies were respon-



sible for the development of cells of this type.

DISCUSSION

The previous studies on saurian malaria have consisted almost exclusively of species descriptions from naturally infected lizards. With the exception of the unsuccessful attempts of Aragão and Neiva (1909) to isolate *P. diplo-*

experimental work were taken from their natural habitats in areas where no malarial parasites have been observed. It seems desirable to consider the evidence indicating that the experimental lizards were uninfected, and that there are certain localities in which no malarial parasites occur in lizards. The importance of the evidence indicating a sporadic geographical distribution of

TABLE 4.—*Species of Plasmodium Described in Lizards*

| Parasites                     | Type Host                         | Type Locality               | References                   |
|-------------------------------|-----------------------------------|-----------------------------|------------------------------|
|                               | Agamidae                          |                             |                              |
| <i>P. agamae</i>              | <i>Agama agama</i>                | Bahr-El-Ghazal, Sudan       | Wenyon (1909)                |
| <i>P. giganteum</i>           | <i>Agama agama</i>                | Ghanga, Liberia             | Theiler (1930)               |
|                               | Anguidae                          |                             |                              |
| <i>P. diploglossi</i>         | <i>Diploglossus fasciatus</i>     | Minas Geraes, Brazil        | Aragão and Neiva (1909)      |
|                               | Geckonidae                        |                             |                              |
| <i>P. simondi</i> *           | <i>Hemidactylus leschenaultii</i> | Northern Province, Ceylon   | Castelani and Willey, (1904) |
|                               | Iguanidae                         |                             |                              |
| <i>P. carinii</i>             | <i>Iguana delicatissima</i>       | French Guiana               | Leger and Mouzels (1917)     |
| <i>P. floridense</i> , n. sp. | <i>Sceloporus undulatus</i>       | Ocala, Florida              | (this paper)                 |
| <i>P. gonzalet</i> *          | <i>Anolis biporcatus</i>          | Venezuela                   | Iturbe and Gonzalez (1921)   |
| <i>P. mexicanum</i> , n. sp.  | <i>Sceloporus ferrariperezi</i>   | Michoacan, Mexico           | (see preceding paper, 1944)  |
| <i>P. rhadinurum</i> , n. sp. | <i>Iguana iguana rhinolopha</i>   | Colima, Mexico              | (this paper)                 |
| <i>P. stenocerci</i>          | <i>Stenocercus</i> sp.            | Goyaz, Brazil               | Carini (1941)                |
| <i>P. tropiduri</i>           | <i>Tropidurus torquatus</i>       | Minas Geraes, Brazil        | Carini and Rudolph (1912)    |
|                               | Scincidae                         |                             |                              |
| <i>P. mabuia</i>              | <i>Mabuya quinquelaeniata</i>     | Bahr El-Ghazal, Sudan       | Wenyon (1909)                |
| <i>P. maculilabre</i>         | <i>Mabuya maculilabris</i>        | Stanleyville, Belgian Congo | Schwetz (1931)               |
| <i>P. minasense</i>           | <i>Mabuya mabouya</i>             | Minas Geraes, Brazil        | Carini and Rudolph (1912)    |
| <i>P. pitmani</i>             | <i>Mabuya striata</i>             | Sese Islands and Bougerere, | Hoare (1932)                 |
|                               | <i>Mabuya maculilabris</i>        | Uganda                      |                              |

\* Placed in the Genus *Haemoproteus* by Wenyon (1926).

*glossi*, the first experimental work on saurian malaria was that of Huff (1941). The studies presented here represent a continuation of this work. In view of the small amount of emphasis placed on saurian malaria a surprisingly large number of species of *Plasmodium* have been described. The species of *Plasmodium* that have been described in lizards, along with those described in this study, are listed in table 4. Although there have been very few studies on the natural incidence of malarial parasites in lizards, there is substantial evidence that these parasites have a more limited geographical distribution and narrower host range than those in avian hosts. Since lizards raised under controlled conditions have not been available, the animals used for the

these parasites involves not only the justification for using wild lizards for experimental purposes in the present study, but also in determining whether they may be used satisfactorily in future studies. First, there is the evidence from surveys of the natural incidence of *Plasmodium* in various species of lizards. In the present study, *Plasmodium* has been found in only 5 of the 16 localities known to be represented. In other studies, approximately 300 lizards from California were examined with infected animals being encountered in only 3 of the 31 localities, although in one of these 10% of the lizards were infected (Wood and Wood, 1936). In a study of 40 iguanid lizards collected in Mexico, D. F., and near Cuautla, Morelos,

Mexico, all were negative for blood protozoa (unpublished study of Hegner and Hewitt, 1943).

Second, evidence indicating the occurrence of endemic and non-endemic areas is provided in studies of the same species of lizard in different localities. At the time *P. tropiduri* was described in *Tropidurus torquatus*, Aragão and Neiva (1909) concluded that the parasite is not widespread. It occurred in almost every lizard of this species examined from Bicudos, State of Minas Geraes, Brazil, but did not occur in a great number of these lizards from Rio de Janeiro, Xerem, Juiz de Fora, and São Paulo. In the present study, *S. undulatus* was found to harbor *Plasmodium* in only 1 of the 5 localities from which these lizards were examined. The susceptibility of this host, however, is indicated by the fact that 23% of the lizards in the endemic area were infected, as were over half of the specimens of *A. carolinensis* obtained from Ocala, Florida. None of those from 2 localities in Louisiana appeared to be infected.

Third, several species of apparently uninfected lizards have been found to be susceptible to one or more species of malarial parasites. Five species were susceptible to *P. mexicanum*. One species, *S. undulatus*, was susceptible to *P. mexicanum*, *P. rhadinurum*, and *P. floridense*. *C. collaris*, *C. wislizenii*, and *A. carolinensis* have been infected with *P. floridense*. All of the experimental infections followed blood or tissue inoculations, and probably infections could not be maintained in some of these lizards by their invertebrate hosts in nature. This seems to apply particularly to *P. mexicanum* in *C. collaris* and probably to this parasite in *Phrynosoma cornutum*, and *P. asio* since considerable numbers of gametocytes are probably essential to this type of transmission. In the other

instances there is no obvious obstacle to the transmission of the parasites in a natural manner. The fact that these susceptible hosts were uninfected indicates that, at least for the lizards studied, there are *Plasmodium*-free areas.

Malarial parasites of lizards appear to have a relatively narrow host range. This is indicated in the studies on the natural incidence of parasites, and by our attempts to establish experimental infections (see pp. 68, 71) and also by those of Aragão and Neiva (1909) and Huff (1941). *P. mexicanum* seems to have a much narrower host range than *P. elongatum*, the avian parasite to which it is most similar, since it appears to be restricted to the family Iguanidae, while *P. elongatum* has been described in at least 6 families of birds. Lizards of the family Iguanidae have been found infected most frequently. Seven of the 13 apparently valid species of malarial parasites in lizards have been described in this family, although according to Camp (1923) there are 21 families of lizards.

The apparently limited geographical and host distribution of *Plasmodium* in lizards is probably partly due to the restricted dispersal range of lizards. Herpetologists have observed that lizards tend to remain in a given area; in fact, knowledge of the locality in which a particular specimen has been found is an especially valuable aid in the identification of lizards. A simple explanation for the limited geographical and host distribution of the parasites may be that, in addition to the limited rate of dispersal of lizards, the environmental conditions in various localities are unfavorable for the development and survival of the vectors of malarial parasites in lizards. Among the various factors controlling the rate of evolution in a population, isolation provides es-



pecially favorable conditions for the development of new species and for specialization such as is reflected in a narrow host range. For these reasons it seems probable that further study of saurian malaria will reveal more species of *Plasmodium* than have been described in birds.

The present work has dealt with only a few of the aspects of saurian malaria. This is a fruitful field for investigation and further study of these infections should add to the fundamental understanding of various phases of malariology. The use of the lizard as an experimental animal has certain advantages as compared with other animals. For instance, a large number of species may be procured easily from professional collectors, lizards are relatively inexpensive, and they may be maintained in the laboratory at little expense. In addition, since they are cold-blooded animals and can survive for many days without food it is possible to control the physiology of these hosts to an extent not possible in warm-blooded animals. However, certain disadvantages are also associated with the use of lizards as experimental animals. Probably the most important of these is that a relatively long time is required for infections to develop and run their full course, and before they can be used extensively in research, standardized procedures must be developed. In addition, considerable experience is required for maintaining them in the laboratory, and special technics must be utilized in studying and maintaining malarial infections in this type of host.

#### SUMMARY AND CONCLUSIONS

*Plasmodium rhadinurum* n. sp. was isolated from the green iguana, *Iguana iguana rhinolopha*, obtained from Colima, Mexico, by injecting blood into *Sceloporus undulatus*.

*Plasmodium floridense*, n. sp., has been described from *Sceloporus undulatus* obtained from Ocala, Florida. It has been isolated and studied in experimentally infected lizards of the same species from other localities. This parasite has an asynchronous schizogony.

The natural incidence of blood parasites is described in 1,136 North and Central American lizards. Approximately 9.4% were infected with malarial parasites, 2.7% with unidentified sporozoa, and 1.1% with microfilariae.

The sporadic geographical distribution of malarial parasites in lizards is described and discussed and evidence is presented indicating that these parasites have a relatively narrow host range.

#### REFERENCES

- Aragão, H. de B. and Neiva, A. (1909) Mem. Inst. Oswaldo Cruz. **1**: 44.  
 Bloom, Wm. (1943) Personal communication.  
 Brumpt, E. (1910) Précis de Parasitologie. Paris.  
 Camp, C. L. (1923) Bull. Amer. Mus. Nat. Hist. **48**: 289.  
 Cannon, P. R. and Taliaferro, W. H. (1931) J. Prev. Med. **5**: 37.  
 Carini, A. (1941) Arq. de Biol. **25**: 46.  
 Carini, A. and Rudolph, M. (1912) Bull. Soc. path. exot. **5**: 592.  
 Castellani, A. and Willey, A. (1904) Quart. J. Micr. Sci. **49**: 383.  
 Gambrell, W. E. (1937) Amer. J. Trop. Med. **17**: 5.  
 Gordon, F. B. (1943) Personal communication.  
 Hewitt, R. (1943) Personal communication.  
 Hoare, C. A. (1932) Parasit. **24**: 210.  
 Huff, C. G. (1938) Quart. rev. Biol. **13**: 196.  
 Huff, C. G. (1939) Jour. Amer. Vet. Med. Assoc. **94**: 615.  
 Huff, C. G. (1941) J. Parasitol. **27** Suppl.: 29.  
 Huff, C. G. (1942) J. Infect. Dis. **71**: 18.  
 Huff, C. G. and Bloom, W. (1935) J. Infect. Dis. **57**: 315.  
 Iturbe, J. and Gonzalez, E. (1921) Gaceta Med. Caracas **28**: 275.  
 Jordan, H. E. and Speidel, C. C. (1929) Amer. J. Anat. **43**: 77.  
 Laveran, A., and Marullaz, M. (1914) Bull. Soc. path. exot. **7**: 21.

- Leger, M., and Mouzels, P. (1917) Bull. Soc. path. exot. **10**: 95.
- Manwell, R. D. (1936) Ann. Trop. Med. and Parasit. **30**: 435.
- Porter, R. J. (1942) J. Infect. Dis. **71**: 1.
- Porter, R. J., and Huff, C. G. (1940) Amer. J. trop. Med. **20**: 869.
- Rodhain, J. (1938a) Comp. rend. Soc. biol. **127**: 368; (1938b) Comp. rend. Soc. biol. **127**: 838.
- Rodhain, J. (1939) Ann. de Parasit. **17**: 139.
- Schwetz, J. (1931) Ann. de Parasit. **9**: 193.
- Taliaferro, W. H. and Cannon, P. R. (1936) J. Infect. Dis. **59**: 72.
- Taliaferro, W. H. and Mulligan, H. W. (1937) Ind. Med. Res. Mem. **29**: 1
- Taliaferro, W. H. and Taliaferro, L. G. (1934a) Amer. J. Hyg. **19**: 318; (1934b) Amer. J. Hyg. **20**: 1.
- Theiler, M. (1930) The African Republic of Liberia and the Belgian Congo, Cambridge, Vol. I, 568 pp. (See pp. 490-498.)
- Thompson, P. E. and Huff, C. G. (1942) J. Parasitol. **28** Suppl.: 15.
- Wenyon, C. M. (1909) 3rd Rept. Wellcome Trop. Res. Labs., Khartoum. 121.
- Wenyon, C. M. (1915) J. Trop. Med. and Hyg. **18**: 133.
- Wenyon, C. M. (1926) Protozoology. 2 vols. London. (See p. 902.)
- Wolfson, F. (1939) Amer. J. Hyg. **30c**: 123.
- Wolfson, F. (1940) Amer. J. Hyg. **31c**: 26.
- Wood, S. F. and Wood, F. D. (1936) J. Parasitol. **22**: 519.









